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(54) Title: N-SUBSTITUTED GLYCINE DERIVATIVES AS INHIBITORS OF FACTOR Xa

(57) Abstract

The present invention discloses peptide aldehydes which are potent inhibitors of factor Xa, their pharmaceutically acceptable salts, pharmaceutically acceptable compositions thereof, and methods of using them as therapeutic agents for disease states in mammals characterized by abnormal thrombosis.

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DESCRIPTION

N-substituted Glycine Derivatives as Inhibitors of Factor Xa

Cross Reference to Related Application

This application is a Continuation-in-Part of U.S.S.N. 08/484,509, filed June 7, 1995, a Continuation-in-Part of USSN 08/361,794, filed December 21, 1994, the disclosures of which are incorporated herein by reference.

Technical Fields

In one aspect, the present invention relates to compounds which are potent inhibitors of factor Xa. In another aspect, the present invention relates to novel peptide aldehydes, their pharmaceutically acceptable salts, and pharmaceutically acceptable compositions thereof which are useful as potent inhibitors of blood coagulation in vitro and in vivo in mammals. In yet another aspect, the invention relates to methods of using these inhibitors as therapeutic agents for disease states in mammals characterized by abnormal thrombosis. In a further aspect, the invention relates to methods of using these inhibitors as in vitro diagnostic agents.

Background

Normal hemostasis is the result of a delicate balance between the processes of clot formation (blood coagulation) and clot dissolution (fibrinolysis). The complex interactions between blood cells, specific plasma proteins and the vascular surface, maintain the fluidity of blood unless injury occurs. Damage to the endothelial barrier lining the vascular wall exposes underlying tissue to these blood components. This in turn triggers a series of biochemical reactions altering the hemostatic balance in favor of blood coagulation which can either result in the desired formation of a hemostatic plug stemming the loss of blood or the undesirable formation of an occlusive

intravascular thrombus resulting in reduced or complete lack of blood flow to the affected organ.

The blood coagulation response is the culmination of a series of amplified reactions in which several specific zymogens of serine proteases in plasma are activated by limited proteolysis. This series of reactions results in the formation of an insoluble matrix composed of fibrin and cellular components which is required for the stabilization of the primary hemostatic plug or thrombus. The initiation and propagation of the proteolytic activation reactions occurs through a series of amplified pathways which are localized to membranous surfaces at the site of vascular injury (Mann, K.G., Nesheim, M.E., Church, W.R., Haley, P. and Krishnaswamy, S. (1990) Blood 76: 1-16. and Lawson, J.H., Kalafatis, M., Stram, S.,and Mann, K.G. (1994) J. Biol. Chem. 269: 23357-23366).

Initiation of the blood coagulation response to vascular injury follows the formation of a catalytic complex composed of serine protease factor VIIa and the non-enzymatic co-factor, tissue factor (TF)(Rappaport, S.I. and Rao, L.V.M. (1992) Arteriosclerosis and Thrombosis 12: 1112-1121). This response appears to be exclusively regulated by the exposure of subendothelial TF to trace circulating levels of factor VIIa and its zymogen factor VII, following a focal breakdown in vascular integrity. Autoactivation results in an increase in the number of factor VIIa/TF complexes which are responsible for the formation of the serine protease factor Xa. It is believed that in addition to the factor VIIa/TF complex, the small amount of factor Xa which is formed primes the coagulation response through the proteolytic modification of factor IX to factor IX_{alpha} which in turn is converted to the active serine protease factor IXa_b by the factor VIIa/TF complex (Mann, K.G., Krishnaswamy, S. and Lawson, J.H. (1992) Sem. Hematology 29: 213-226.). It is factor IXa_b in complex with activated factor VIIIa, which appears to be responsible for the production of significant quantities of factor Xa which subsequently catalyzes the

penultimate step in the blood coagulation cascade; the formation of the serine protease thrombin.

Factor Xa catalyzes the formation of thrombin following the assembly of the prothrombinase complex which is composed of factor Xa, the non-enzymatic co-factor Va and the substrate prothrombin (factor II) assembled in most cases, on the surface of activated platelets which are adhered at the site of injury (Fuster, V., Badimon, L., Badimon, J.J. and Chesebro, J.H. (1992) New Engl. J. Med. 326: 310-318). In the arterial vasculature, the resulting amplified "burst" of thrombin generation catalyzed by prothrombinase results locally high levels of this protease which is responsible for the formation of fibrin and the further recruitment of additional platelets as well as the covalent stabilization of the clot through the activation of the transglutaminase zymogen factor XIII. In addition, the coagulation response is further propagated through the thrombin-mediated proteolytic feedback activation of the non-enzymatic co-factors V and VIII resulting in more prothrombinase formation and subsequent thrombin generation (Hemker, H.C. and Kessels, H. (1991) Haemostasis 21: 189-196).

Substances which interfere in the process of blood coagulation (anticoagulants) have been demonstrated to be important therapeutic agents in the treatment and prevention of thrombotic disorders (Kessler, C.M. (1991) Chest 99: 97S-112S and Cairns, J.A., Hirsh, J., Lewis, H.D., Resnekov, L., and Theroux, P. (1992) Chest 102: 456S-481S). The currently approved clinical anticoagulants have been associated with a number of adverse effects owing to the relatively non-specific nature of their effect on the blood coagulation cascade (Levine, M.N., Hirsh, J., Landefeld, S., and Raskob, G. (1992) Chest 102: 352S-363S). This has stimulated the search for more effective anticoagulant agents which can more effectively control the activity of the coagulation cascade by selectively interfering with specific reactions in this process which may have a positive effect in

reducing the complications of anticoagulant therapy (Weitz, J., and Hirsh, J. (1993) J. Lab. Clin. Med. 122: 364-373). In another aspect, this search has focused on normal human proteins which serve as endogenous anticoagulants in controlling the activity of the blood coagulation cascade. In addition, various hematophageous organisms have been investigated because of their ability to effectively anticoagulate the blood meal during and following feeding on their hosts suggesting that they have evolved effective anticoagulant strategies which may be useful as therapeutic agents.

A plasma protein, Lipoprotein-Associated Coagulation Inhibitor (LACI) or recently termed Tissue Factor Pathway Inhibitor (TFPI), containing three consecutive Kunitz domains has been reported to inhibit the enzyme activity of factor Xa directly and, in a factor Xa-dependent manner, inhibit the enzyme activity the factor VIIa-tissue factor complex. Salvensen,G., and Pizzo, S.V., "Proteinase Inhibitors: a-Macroglobulines, Serpins, and Kunis", "Hemostasis and Thrombosis, Third Edition, pp. 251-253, J.B. Lippincott Company (Edit. R.W. Colman et al. 1994). A cDNA sequence encoding TFPI has been reported, and the cloned protein was reported to have a molecular weight of 31,950 daltons and contain 276 amino acids. Broze, G.J. and Girad, T.J., U.S. Patent No. 5,106,833, col. 1, (1992). Various recombinant proteins derived from TFPI have been reported. Girad, T.J. and Broze, G.J., EP 439,442 (1991); Rasmussen, J.S. and Nordfand, O.J., WO 91/02753 (1991); and Broze, G.J. and Girad, T.J., U.S. Patent No. 5,106,833, col. 1, (1992).

Antistasin, a protein comprised of 119 amino acids and found in the salivary gland of the Mexican leech, Haementeria officinalis, has been reported to inhibit the enzyme activity of factor Xa. Tuszynski et al. (1987) J. Biol. Chem, 262:9718; Nutt, et al. (1988) J. Biol. Chem, 263:10162. A 6,000 daltons recombinant protein containing 58 amino acids with a high degree homology to antistasin's amino-terminus amino acids 1 through 58 has been reported

to inhibit the enzyme activity of factor Xa. Tung, J. et al., EP 454,372 (1991); Tung, J. et al., U.S. Patent No. 5,189,019 (1993).

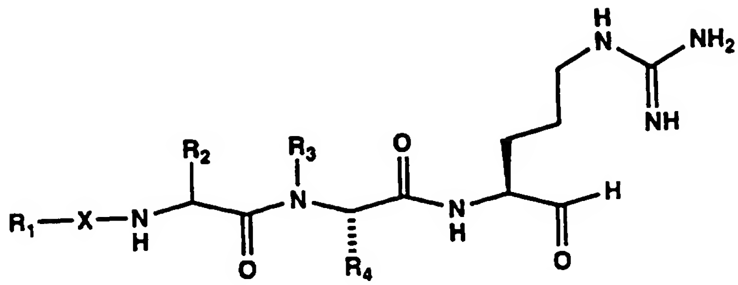
Tick Anticoagulant Protein (TAP), a protein comprised of 60 amino acids and isolated from the soft tick, Ornithodoros moubata, has been reported to inhibit the enzyme activity of factor Xa but not factor VIIa. Waxman, L. et al. (1990) Science, 248:593. TAP made by recombinant methods has been reported. Vlausk, G.P. et al., EP 419,099 (1991) and Vlausk, G.P. et al., U.S. Patent No 5,239,058 (1993).

The dog hookworm, Ancylostoma caninum, which can also infect humans, has been reported to contain a potent anticoagulant substance. A. caninum was reported to contain a substance which inhibited coagulation of blood in vitro. Loeb, L. and Smith, A.J. (1904) Proc. Pathol. Soc. Philadelphia, 7:173-178. Extracts of A. caninum were reported to prolong prothrombin time and partial thromboplastin time in human plasma with the anticoagulant effect being reported attributable to inhibition of factor Xa but not thrombin. Spellman, Jr., J.J. and Nossel, H.L. (1971) Am. J. Physiol., 220:922-927. More recently, soluble protein extracts of A. caninum were reported to prolong prothrombin time and partial thromboplastin time in human plasma in vitro. The anticoagulant effect was reported to be attributable to inhibition of human factor Xa but not thrombin. Cappello, M, et al. (1993) J. Infect. Diseases, 167:1474-1477.

Summary of the Invention

The present invention is directed to novel compounds which are peptide argininals which include N-substituted glycine groups as part of the peptide backbone. These compounds are potent inhibitors of factor Xa *in vivo* and *in vitro*.

Thus, in one aspect, the present invention is directed to compounds of the formula:



(I)

wherein

(a) X is selected from the group consisting of $-S(O)_2-$, $-N(R')-S(O)_2-$, $-(C{=}O)-$, $-OC(=O)-$, $-NH-C(=O)-$, $-P(O)(R")-$ and a direct link, wherein R' is hydrogen, alkyl of 1 to about 4 carbon atoms, aryl of about 6 to about 14 carbon atoms or aralkyl of about 6 to about 16 carbon atoms, and R" is NR', OR', R', or SR', with the proviso that R" is not NH, OH, H, or SH, and ;

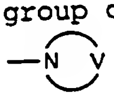
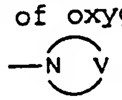
(b) R_1 is selected from the group consisting of:

(1) alkyl of 1 to about 12 carbon atoms optionally substituted with Y_1,

(2) alkyl of 1 to about 3 carbon atoms substituted with cyclic alkyl of about 5 to about 8 carbon atoms optionally substituted on the ring with Y_1, Y_2 and/or Y_3,

(3) cyclic alkyl of 3 to about 15 carbon atoms, which optionally is substituted on the ring with Y_1, Y_2 and/or Y_3,

(4) heterocycloalkyl of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and $S(O)_i$, wherein i is 0, 1 or 2, optionally substituted on the ring with Y_1, Y_2 and/or Y_3,

(5) heterocyclo of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and $S(O)_i$, including , wherein  is a 5 to 7 member heterocycle of 3 to 6 ring carbon atoms, where V is $-CH_2-$, $-O-$, $-S(=O)-$,

$-S(O)_2-$ or -S-, and which is optionally substituted on the ring carbons with Y_1, Y_2 and/or Y_3,

(6) alkenyl of about 2 to about 6 carbon atoms which is optionally substituted with cyclic alkyl of about 5 to about 8 carbon atoms, which optionally is substituted on the ring carbons with Y_1, Y_2 and/or Y_3,

(7) aryl of about 6 to about 14 carbon atoms which is optionally mono-, di- or tri-substituted with Y_1, Y_2, and/or Y_3, respectively,

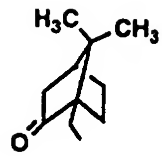
(8) heteroaryl of 5 to 14 atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and $S(O)_i$, and which is optionally mono-, di- or tri-substituted with Y_1, Y_2, and/or Y_3, respectively,

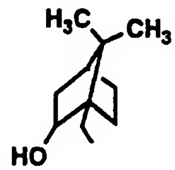
(9) aralkyl of about 7 to about 15 carbon atoms which is optionally substituted on the alkyl chain with hydroxy or halogen and mono-, di-, or tri-substituted in the aryl ring with Y_1, Y_2, and/or Y_3, respectively,

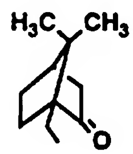
(10) heteroaralkyl of 6 to 11 atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and $S(O)_i$, and which is optionally substituted on the alkyl chain with hydroxy or halogen and optionally mono-, di- or tri-substituted on the ring with Y_1, Y_2, and/or Y_3, respectively,

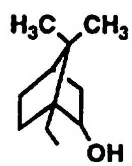
(11) aralkenyl of about 8 to about 15 carbon atoms which is optionally mono-, di-, or tri-substituted in the aryl ring with Y_1, Y_2, and/or Y_3, respectively,

(12) heteroaralkenyl of 7 to 12 atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and $S(O)_i$, and which is optionally mono-, di- or tri-substituted on the ring with Y_1, Y_2, and/or Y_3, respectively,

(13)  ,

(14)  ,

(15)  ,

(16)  ,

(17) difluoromethyl or perfluoroalkyl of 1 to about 12 carbon atoms,

(18) perfluoroaryl of about 6 to about 14 carbon atoms,

(19) perfluoroaralkyl of about 7 to about 15 carbon atoms, and

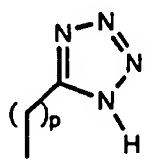
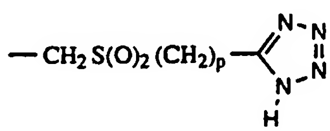
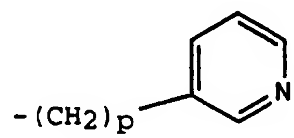
(20) hydrogen,

wherein Y_1, Y_2, and Y_3 are

(i) independently selected from the group consisting of halogen, cyano, nitro, tetrazolyl, amino, guanidino, amidino, methylamino, and methylguanidino, $-CF_3$, $-CF_2CF_3$, $-CH(CF_3)_2$, $-C(OH)(CF_3)_2$, $-OCF_3$, $-OCF_2CF_3$, $-OC(O)NH_2$, $-OC(O)NHZ_1$, $-OC(O)NZ_1Z_2$, $-NHC(O)Z_1$, $-NHC(O)NH_2$, $-NHC(O)NZ_1$, $-NHC(O)NZ_1Z_2$, $-C(O)OH$, $-C(O)OZ_1$, $-P(O)_3H$, $-P(O)_3H_2$, $-P(O)_3(Z_1)_2$, $-S(O)_3H$, $-S(O)_mZ_1$, $-Z_1$, $-OZ_1$, $-OH$, $-NH_2$, $-NHZ_1$, $-NZ_1Z_2$, and N-morpholino, wherein m is 0, 1 or 2, and Z_1 and Z_2 are independently selected from the group consisting of alkyl of 1 to about 12 carbon atoms, aryl of about 6 to about 14 carbon atoms, heteroaryl of about 5 to about 14 atoms

having 1 to about 9 carbon atoms, aralkyl of about 7 to about 15 carbon atoms, and heteroaralkyl of about 6 to about 11 atoms having about 3 to about 9 carbon atoms, or

(ii) Y_1 and Y_2 are selected together to be $-OC(Z_3)(Z_4)O-$, wherein Z_3 and Z_4 are independently selected from the group consisting of hydrogen, alkyl of 1 to about 12 carbon atoms, aryl of about 6 to about 14 carbon atoms, heteroaryl of about 5 to about 14 atoms having 1 to about 9 carbon atoms, aralkyl of about 7 to about 15 carbon atoms, and heteroaralkyl of about 6 to about 11 atoms having about 3 to about 9 carbon atoms, with the proviso that if X is not a direct link, then R_1 is not hydrogen,

(c) R_2 is selected from the group consisting of , , , hydrogen, $-(CH_2)_pNHC(=NH)NH_2$, $-(CH_2)_pS(O)_2CH_3$, $-(CH_2)_pC(O)Z_5$, $-(CH_2)_pC(O)OZ_6$, $-(CH_2)_pC(O)NZ_6$, $-CH_2S(O)_2(CH_2)_pC(O)Z_5$, $-CH_2S(O)_2(CH_2)_pC(O)OZ_6$, $-CH_2S(O)_2(CH_2)_pC(O)NR_5R_6$, $-(CH_2)_pS(O)_2Z_6$, $-(CH_2)_pNH_2$, $-(CH_2)_pC(O)NR_5R_6$, $-(CH_2)_pC(O)Z_6$, $-(CH_2)_pOZ_6$ and $-(CH_2)_pC(O)$ wherein

p is an integer from 1 to 6,

Z_5 is $-OH$, $-OCH_3$, $-OCH_2CH_3$, or $-NR_5R_6$,

Z_6 is alkyl of 1 to about 4 carbon atoms, aryl of about 6 to about 14 carbon atoms, or aralkyl of about 7 to 16 carbon atoms,

R_5 is hydrogen, or Z_6,

R_6 is hydrogen or cyclic alkyl of 3 to about 15 carbon atoms optionally mono- di- or tri-substituted with Y_1, Y_2 and/or Y_3, aralkyl of about 7 to about 15 carbon atoms optionally mono-, di- or tri-substituted with Y_1, Y_2 and/or Y_3, heteroaryl of 5 to 14 atoms with the ring atoms selected from carbon and heteroatoms wherein the heteroatoms are selected from oxygen, nitrogen, and $S(O)_i$, and which is optionally mono-, di- or tri-substituted with

Y_1, Y_2, and/or Y_3, quinuclidine, or adamantyl,

 is 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolinyl, 4-hydroxy piperidyl, 4-keto piperidyl, N-morpholino, 3,4-methylenedioxybenzyl piperazinyl, 4-phenyl piperazinyl optionally mono-substituted with fluoro, chloro, methoxy, or trifluoromethyl, or 4-benzyl piperazinyl optionally mono-substituted with fluoro, chloro, methoxy, or trifluoromethyl,

and pharmaceutically acceptable quaternary ammonium salts thereof;

(d) R_3 is selected from the group consisting of

(1) hydrogen;

(2) alkyl of 1 to about 8 carbon atoms optionally substituted with -OH;

(3) cyclic alkyl of about 3 to about 10 carbon atoms;

(4) alkyl of 1 to about 3 carbon atoms substituted with cyclic alkyl of about 5 to about 8 carbon atoms;

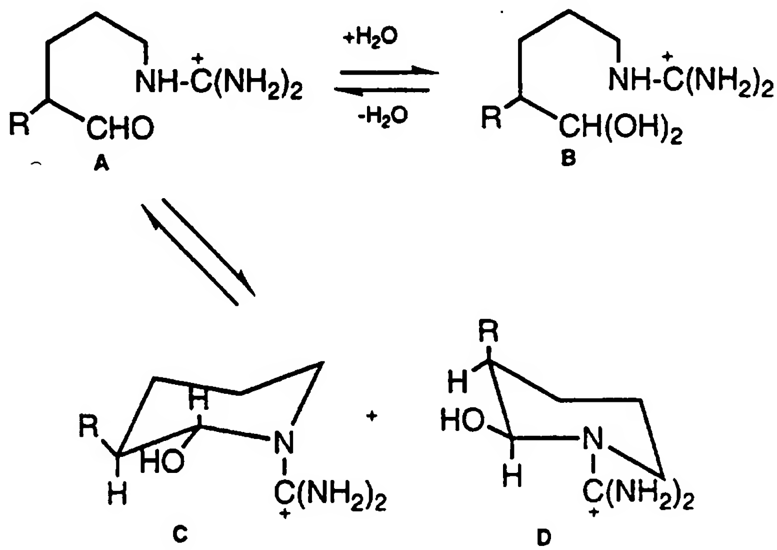
(5) aryl of about 3 to about 10 carbon atoms which is optionally mono-, di-, or tri-substituted with Y_1, Y_2 and/or Y_3;

(6) alkyl of 1 to about 3 carbon atoms substituted on the terminal carbon with aryl of about 4 carbon atoms to about 10 carbon atoms which is optionally mono-, di-, or tri-substituted with Y_1, Y_2 and/or Y_3;

(7) alkyl of 1 to about 6 carbon atoms with alkyl branching at the alpha, beta, gamma, and delta carbons of 1 to about 6 carbon atoms; and

(e) R_4 is selected from the group consisting of hydrogen, alkyl of 1 to about 7 carbon atoms optionally substituted with -OH or benzyloxy and alkyl of 1 to about 3 carbon atoms substituted on the terminal carbon atom with aryl of about 4 carbon atoms to about 10 carbon atoms which is optionally mono-, di-, or tri-substituted with Y_1, Y_2 and/or Y_3.

Peptidyl arginine aldehydes have been reported to exist in equilibrium structures in aqueous solutions. Bajusz, S., et al. (1990) J. Med. Chem., 33: 1729. These structures, as shown below, include the arginine aldehyde, A, aldehyde hydrate, B, and two amino cyclol forms, C and D. The R group would represent the remainder of a given compound embodied in the present invention. The peptide aldehydes of the present invention include within their definition all the equilibrium forms.



Among other factors, the present invention is based on our finding that the novel compounds of our invention are active as potent inhibitors of factor Xa in vivo and in vitro. In particular, we have found that certain of the preferred compounds of the present invention exhibit advantageous selectivity in that they are very potent inhibitors of factor Xa but are inactive or significantly less active, (several orders of magnitude less) in inhibiting plasmin and are significantly less active in inhibiting thrombin.

In another aspect, the present invention is directed to pharmaceutical compositions comprising a therapeutically effective amount of a compound of the present invention and a pharmaceutically acceptable carrier.

In yet another aspect, the present invention is

direct d to methods of using the compounds and pharmaceutical compositions of the present invention for the prevention of thrombosis in a mammal suspected of having a condition characterized by abnormal thrombosis, comprising administering to said mammal a therapeutically effective amount of a compound of the present invention or pharmaceutical composition comprising such a compound.

Definitions

In accordance with the present invention and as used herein, the following terms are defined to have following meanings, unless explicitly stated otherwise:

The term "alkenyl" refers to unsaturated aliphatic groups having at least one double bond.

The term "alkyl" refers to saturated aliphatic groups including straight-chain, branched-chain and cyclic groups.

The terms "alkoxy" and "alkoxyl" refer to a group having the formula, R-O-, wherein R is an alkyl group.

The term "alkoxycarbonyl" refers to -C(O)OR wherein R is alkyl.

The term "aralkenyl" refers to an alkenyl group substituted with an aryl group.

The term "aralkyl" refers to an alkyl group substituted with an aryl group. Suitable aralkyl groups include benzyl, picolyl, and the like, all of which may be optionally substituted.

The term "aryl" refers to aromatic groups which have at least one ring having a conjugated pi electron system and includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted.

The term "aryloxy" refers to a group having the formula, R-O-, wherein R is an aryl group.

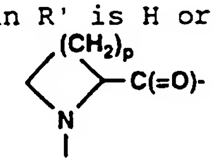
The term "aralkoxy" refers to a group having the formula, R-O-, wherein R is an aralkyl group.

The term "amino acid" refers to both natural, unnatural amino acids in their D and L stereoisomers if their structure allow such stereoisomeric forms, and their analogs. Natural amino acids include alanine (Ala),

arginine (Arg), asparagine (Asn), aspartic acid (Asp), cysteine (Cys), glutamine (Gln), glutamic acid (Glu), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tryptophan (Trp), tyrosine (Tyr) and valine (Val). Unnatural amino acids include, but are not limited to azetidinecarboxylic acid, 2-aminoadipic acid, 3-aminoadipic acid, beta-alanine, aminopropionic acid, 2-aminobutyric acid, 4-aminobutyric acid, 6-aminocaproic acid, 2-aminoheptanoic acid, 2-aminoisobutyric acid, 3-aminoisobutyric acid, 2-aminopimelic acid, 2,4 diaminoisobutyric acid, desmosine, 2,2'-diaminopimelic acid, 2,3-diaminopropionic acid, N-ethylglycine, N-ethylasparagine, hydroxylysine, allo-hydroxylysine, 3-hydroxyproline, 4-hydroxyproline, isodesmosine, allo-isoleucine, N-methylglycine, N-methylisoleucine, N-methylvaline, norvaline, norleucine, ornithine and pipecolic acid. Amino acid analogs include the natural and unnatural amino acids which are chemically blocked, reversibly or irreversibly, or modified on their N-terminal amino group or their side-chain groups, as for example, methionine sulfoxide, methionine sulfone, S-(carboxymethyl)-cysteine, S-(carboxymethyl)-cysteine sulfoxide and S-(carboxymethyl)-cysteine sulfone.

The term "amino acid analog" refers to an amino acid wherein either the C-terminal carboxy group, the N-terminal amino group or side-chain functional group has been chemically modified to another functional group. For example, aspartic acid-(beta-methyl ester) is an amino acid analog of aspartic acid; N-ethylglycine is an amino acid analog of glycine; or alanine carboxamide is an amino acid analog of alanine.

The term "amino acid residue" refers to radicals having the structure: (1) -C(O)-R-NH-, wherein R typically is -CH(R')-, wherein R' is H or a carbon containing substituent; or (2)



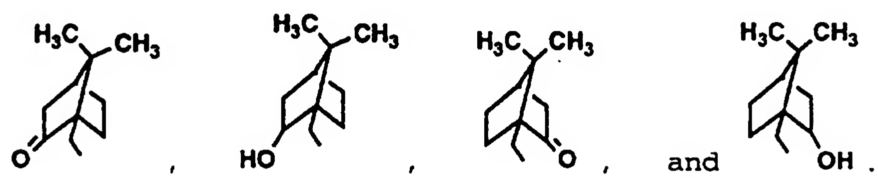
, wherein p is 1, 2 or 3

representing the azetidinecarboxylic acid, proline or pipecolic acid residues, respectively.

"Biaryl" refers to phenyl substituted by carbocyclic or heterocyclic aryl as defined herein, ortho, meta or para to the point of attachment of the phenyl ring.

"Brine" refers to an aqueous saturated solution of sodium chloride.

"Camphor derivative" refers to the groups:



"Carbocyclic aryl" refers to aromatic groups wherein the ring atoms on the aromatic ring are carbon atoms. Carbocyclic aryl groups include monocyclic carbocyclic aryl groups and naphthyl groups, all of which may be optionally substituted. Suitable carbocyclic aryl groups include phenyl and naphthyl. Suitable substituted carbocyclic aryl groups include indene and phenyl substituted by one to two substituents such being advantageously lower alkyl, hydroxy, lower alkoxy, lower alkoxycarbonyl, halogen, trifluoromethyl, nitro, and cyano. Substituted naphthyl refers to 1- or 2-naphthyl substituted by lower alkyl, lower alkoxy, or halogen.

"Cycloalkenyl" refers to a cyclic alkenyl group. Suitable cycloalkenyl groups include, for example, cyclopentenyl and cyclohexenyl.

"Cycloalkyl" refers to a cyclic alkyl group. Suitable cycloalkyl groups include, for example, cyclohexyl, cyclopropyl, cyclopentyl, and cycloheptyl.

"Cyclohexylmethyl" refers to a cyclohexyl group attached to CH_2.

The term "halogen" refers to fluorine, chlorine, bromine and iodine.

"Heteroaralkenyl" refers to an alkenyl group substitued with a heteroaryl.

"Heteroaralkyl" refers to an alkyl group substituted

with a heteroaryl.

"Heteroaryl" refers to aryl groups having from 1 to 9 carbon atoms and the remainder of the atoms are heteroatoms. Suitable heteroatoms include oxygen, nitrogen, $S(O)_i$, wherein i is 0, 1 or 2, and suitable heterocyclic aryls include furanyl, thienyl, pyridyl, pyrrolyl, pyrimidyl, pyrazinyl, imidazolyl, and the like.

"Heterocyclo" refers to a reduced heterocyclic ring system comprised of carbon, nitrogen, oxygen and/or sulfur atoms.

"Heterocycloalkyl" refers to an alkyl group substituted with a heterocyclo group, and includes those heterocyclic systems described in "Handbook of Chemistry and Physics", 49th edition, 1968, R.C. Weast, editor;The Chemical Rubber Co., Cleveland, OH. See particularly Section C, Rules for Naming Organic Compounds, B. Fundamental Heterocyclic Systems.

The term "lower" referred to herein in connection with organic radicals or compounds defines such with up to and including 5, preferably up to and including 4 and advantageously one or two carbon atoms. Such groups may be straight chain or branched chain.

"Perfluoroalkyl" refers to an alkyl group which has every hydrogen replaced with fluorine.

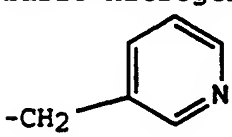
"Perfluoroaryl" refers to an aryl group which has every hydrogen replaced with fluorine.

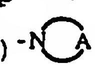
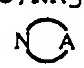
"Perfluoroaryl alkyl" refers an aralkyl group in which every hydrogen on the aryl moiety is replaced with fluorine.

"Pharmaceutically acceptable salt" includes salts of the compounds of the present invention derived from the combination of such compounds and an organic or inorganic acid. In practice the use of the salt form amounts to use of the base form. The compounds of the present invention are useful in both free base and salt form, with both forms being considered as being within the scope of the present invention.

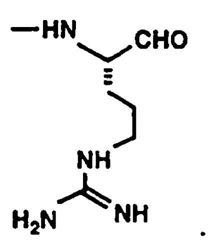
The term "quaternary ammonium salt" refers to

compounds produced by reaction between a basic nitrogen in an R_2 substituent and an alkylhalide, arylhalide, and aralkylhalide. Other reactants with good leaving groups may also be used, such as alkyl trifluoromethanesulfonates, alkyl methanesulfonates, and alkyl p-toluenesulfonates. A quaternary ammonium salt has a positively charged nitrogen in the R_2 substituent. Pharmaceutically acceptable counterions include Cl-, Br-, I-, $CF_3C(O)O$- and $CH_3C(O)O$-. The counterion of choice can be made using ion exchange resin columns. R_2 groups with basic nitrogens include $-CH_2CH_2CH_2NHC(=NH)NH_2$,

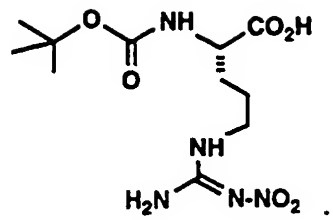


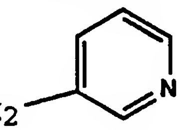
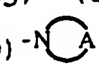
, $-(CH_2)_pNH_2$, wherein p is as defined in conjunction with formula I above. In addition, the following R_2 groups may contain basic nitrogens: $-CH_2S(O)_2(CH_2)_pC(O)Z_5$, $-(CH_2)_pS(O)_2Z_6$, $-(CH_2)_pC(O)NR_5R_6$, and $-(CH_2)_pC(O)$-  ,wherein Z_5, Z_6, R_5, R_6, and  are as defined in conjunction with formula I above. For example, the following R_6 groups contain basic amines: 3-(R)-quinuclidine, 3-(S)-quinuclidine, 3-yl-2-ethyl-4(3H)-quinazolinone, ethyl morpholine, ethyl piperidine, 2-(2-ethyl)pyridine, and 4-(methyl)-5-hydroxy-6-methyl-3-pyridine methanol.

The term "Arg-al" refers to the residue of L-argininal which has the formula:



The term "N-alpha-t-butoxycarbonyl-N^g-nitro-L-arginine" refers to the compound which has the formula:



R_2 groups with basic nitrogens include $-CH_2CH_2CH_2NHC(=NH)NH_2$, $-CH_2$, $-(CH_2)_pNH_2$, wherein p is as defined in conjunction with formula I above. In addition, the following R_2 groups may contain basic nitrogens: $-CH_2S(O)_2(CH_2)_pC(O)Z_5$, $-(CH_2)_pS(O)_2Z_6$, $-(CH_2)_pC(O)NR_5R_6$, and $-(CH_2)_pC(O)-$, wherein each Z_5, Z_6, R_5, R_6, and  is as defined in conjunction with formula I above. For example, the following R_6 groups contain basic amines: 3-(R)-quinuclidine, 3-(S)-quinuclidine, 3-yl-2-ethyl-4(3H)-quinazolinone, ethyl morpholine, ethyl piperidine, 2-(2-ethyl)pyridine, and 4-(methyl)-5-hydroxy-6-methyl-3-pyridine methanol.

The term "terminal carbon" refers to the carbon atom of a straight chain alkyl which is furthest from the parent structure.

In addition, the following abbreviations stand for the following:

"Boc" or "BOC" refers to t-butoxycarbonyl.

"BOP" refers to benzotriazol-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate.

"$BzlSO_2$" refers to benzylsulfonyl.

"DCC" refers to N,N'-dicyclohexylcarbodiimide.

"EDC" refers to 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride salt.

"HATU" refers to O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate.

"HBTU" refers to 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate.

"HCl" refers to hydrochloric acid.

"HOAt" refers to 1-hydroxy-7-azabenzotriazole.

"HOBt" refers to 1-hydroxybenzotriazole monohydrate.

"HPLC" refers to high pressure liquid chromatography.

"$LiAlH_4$" refers to lithium aluminum hydride.

"$LiAlH_2(OEt)_2$ refers to lithium aluminum dihydride diethoxide.

"NMM" refers to N-methylmorpholine.

"NaOH" refers to sodium hydroxide.

"THF" refers to tetrahydrofuran.

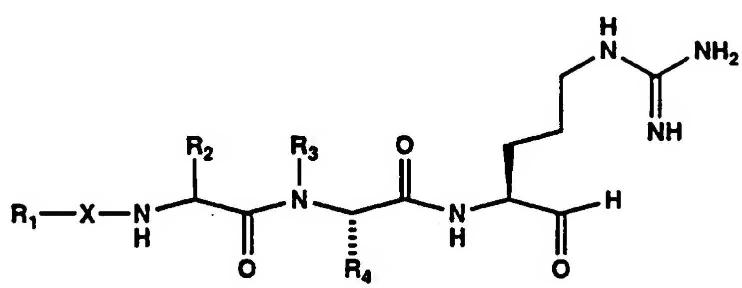
"TBTU" refers to 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate.

"TLC" refers to thin layer chromatography.

Detailed Description of the Invention

1. Preferred Compounds

Compounds of the present invention have the formula



(I)

wherein

(a) X is selected from the group consisting of $-S(O)_2-$, $-N(R')-S(O)_2-$, -(C=O)-, -OC(=O)-, -NH-C(=O)-, -P(O)(R")- and a direct link, wherein R' is hydrogen, alkyl of 1 to about 4 carbon atoms, aryl of about 6 to about 14 carbon atoms or aralkyl of about 6 to about 16 carbon atoms, and R" is NR', OR', R', or SR', with the proviso that R" is not NH, OH, H, or SH, and ;

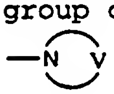
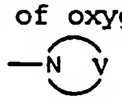
(b) R_1 is selected from the group consisting of:

(1) alkyl of 1 to about 12 carbon atoms optionally substituted with Y_1,

(2) alkyl of 1 to about 3 carbon atoms substituted with cyclic alkyl of about 5 to about 8 carbon atoms optionally substituted on the ring with Y_1, Y_2 and/or Y_3,

(3) cyclic alkyl of 3 to about 15 carbon atoms, which optionally is substituted on the ring with Y_1, Y_2 and/or Y_3,

(4) heterocycloalkyl of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and $S(O)_i$, wherein i is 0, 1 or 2, optionally substituted on the ring with Y_1, Y_2 and/or Y_3,

(5) heterocyclo of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and $S(O)_i$, including , wherein  is a 5 to 7 member heterocycle of 3 to 6 ring carbon atoms, where V is $-CH_2-$, -O-, -S(=O)-, $-S(O)_2-$ or -S-, and which is optionally substituted on the ring carbons with Y_1, Y_2 and/or Y_3,

(6) alkenyl of about 2 to about 6 carbon atoms which is optionally substituted with cyclic alkyl of about 5 to about 8 carbon atoms, which optionally is substituted on the ring carbons with Y_1, Y_2 and/or Y_3,

(7) aryl of about 6 to about 14 carbon atoms which is optionally mono-, di- or tri-substituted with Y_1, Y_2, and/or Y_3, respectively,

(8) heteroaryl of 5 to 14 atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and $S(O)_i$, and which is optionally mono-, di- or tri-substituted with Y_1, Y_2, and/or Y_3, respectively,

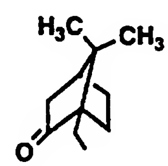
(9) aralkyl of about 7 to about 15 carbon atoms which is optionally substituted on the alkyl chain with hydroxy or halogen and mono-, di-, or tri-substituted in the aryl ring with Y_1, Y_2, and/or Y_3, respectively,

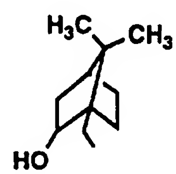
(10) heteroaralkyl of 6 to 11 atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and $S(O)_i$, and which is optionally substituted

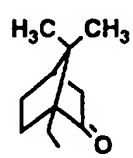
on the alkyl chain with hydroxy or halogen and optionally mono-, di- or tri-substituted on the ring with Y_1, Y_2, and/or Y_3, respectively,

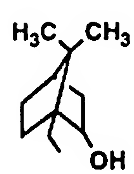
(11) aralkenyl of about 8 to about 15 carbon atoms which is optionally mono-, di-, or tri-substituted in the aryl ring with Y_1, Y_2, and/or Y_3, respectively,

(12) heteroaralkenyl of 7 to 12 atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and $S(O)_i$, and which is optionally mono-, di- or tri-substituted on the ring with Y_1, Y_2, and/or Y_3, respectively,

(13)  ,

(14)  ,

(15)  ,

(16)  ,

(17) difluoromethyl or perfluoroalkyl of 1 to about 12 carbon atoms,

(18) perfluoroaryl of about 6 to about 14 carbon atoms,

(19) perfluoroaralkyl of about 7 to about

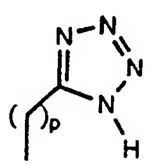
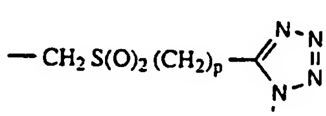
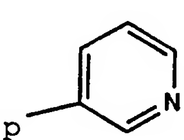
15 carbon atoms, and

(20) hydrogen,

wherein Y_1, Y_2, and Y_3 are

(i) independently selected from the group consisting of halogen, cyano, nitro, tetrazolyl, amino, guanidino, amidino, methylamino, and methylguanidino, $-CF_3$, $-CF_2CF_3$, $-CH(CF_3)_2$, $-C(OH)(CF_3)_2$, $-OCF_3$, $-OCF_2CF_3$, $-OC(O)NH_2$, $-OC(O)NHZ_1$, $-OC(O)NZ_1Z_2$, $-NHC(O)Z_1$, $-NHC(O)NH_2$, $-NHC(O)NZ_1$, $-NHC(O)NZ_1Z_2$, $-C(O)OH$, $-C(O)OZ_1$, $-P(O)_3H$, $-P(O)_3H_2$, $-P(O)_3(Z_1)_2$, $-S(O)_3H$, $-S(O)_mZ_1$, $-Z_1$, $-OZ_1$, $-OH$, $-NH_2$, $-NHZ_1$, $-NZ_1Z_2$, and N-morpholino, wherein m is 0, 1 or 2, and Z_1 and Z_2 are independently selected from the group consisting of alkyl of 1 to about 12 carbon atoms, aryl of about 6 to about 14 carbon atoms, heteroaryl of about 5 to about 14 atoms having 1 to about 9 carbon atoms, aralkyl of about 7 to about 15 carbon atoms, and heteroaralkyl of about 6 to about 11 atoms having about 3 to about 9 carbon atoms, or

(ii) Y_1 and Y_2 are selected together to be $-OC(Z_3)(Z_4)O-$, wherein Z_3 and Z_4 are independently selected from the group consisting of hydrogen, alkyl of 1 to about 12 carbon atoms, aryl of about 6 to about 14 carbon atoms heteroaryl of about 5 to about 14 atoms having 1 to about 9 carbon atoms, aralkyl of about 7 to about 15 carbon atoms, and heteroaralkyl of about 6 to about 11 atoms having about 3 to about 9 carbon atoms, with the proviso that if X is not a direct link, then R_1 is not hydrogen,

(c) R_2 is selected from the group consisting of , , $-(CH_2)_p$, hydrogen, $-(CH_2)_pNHC(=NH)NH_2$, $-(CH_2)_pS(O)_2CH_3$, $-(CH_2)_pC(O)Z_5$, $-(CH_2)_pC(O)OZ_6$, $-(CH_2)_pC(O)NZ_6$, $-CH_2S(O)_2(CH_2)_pC(O)Z_5$, $-CH_2S(O)_2(CH_2)_pC(O)OZ_6$, $-CH_2S(O)_2(CH_2)_pC(O)NR_5R_6$, $-(CH_2)_pS(O)_2Z_6$, $-(CH_2)_pNH_2$, $-(CH_2)_pC(O)NR_5R_6$, $-(CH_2)_pC(O)Z_6$, $-(CH_2)_pOZ_6$ and

$-(CH_2)_pC(O)-N\textcircled{A}$ wherein

p is an integer from 1 to 6,

Z_5 is $-OH$, $-OCH_3$, $-OCH_2CH_3$, or $-NR_5R_6$,

Z_6 is alkyl of 1 to about 4 carbon atoms, aryl of about 6 to about 14 carbon atoms, or aralkyl of about 7 to 16 carbon atoms,

R_5 is hydrogen, or Z_6,

R_6 is hydrogen or cyclic alkyl of 3 to about 15 carbon atoms optionally mono- di- or tri-substituted with Y_1, Y_2 and/or Y_3, aralkyl of about 7 to about 15 carbon atoms optionally mono-, di- or tri-substituted with Y_1, Y_2 and/or Y_3, heteroaryl of 5 to 14 atoms with the ring atoms selected from carbon and heteroatoms wherein the heteroatoms are selected from oxygen, nitrogen, and $S(O)_i$ and which is optionally mono-, di- or tri-substituted with Y_1, Y_2, and/or Y_3, quinuclidine, or adamantyl,

$-N\textcircled{A}$ is 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolinyl, 4-hydroxy piperidyl, 4-keto piperidyl, N-morpholino, 3,4-methylenedioxybenzyl piperazinyl, 4-phenyl piperazinyl optionally mono-substituted with fluoro, chloro, methoxy, or trifluoromethyl, or 4-benzyl piperazinyl optionally mono-substituted with fluoro, chloro, methoxy, or trifluoromethyl,

and pharmaceutically acceptable quaternary ammonium salts thereof;

(d) R_3 is selected from the group consisting of

(1) hydrogen;

(2) alkyl of 1 to about 8 carbon atoms optionally substituted with -OH;

(3) cyclic alkyl of about 3 to about 10 carbon atoms;

(4) alkyl of 1 to about 3 carbon atoms substituted with cyclic alkyl of about 5 to about 8 carbon atoms;

(5) aryl of about 3 to about 10 carbon

atoms which is optionally mono-, di-, or tri-substituted with Y_1, Y_2 and/or Y_3;

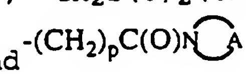
(6) alkyl of 1 to about 3 carbon atoms substituted on the terminal carbon with aryl of about 4 carbon atoms to about 10 carbon atoms which is optionally mono-, di-, or tri-substituted with Y_1, Y_2 and/or Y_3;

(7) alkyl of 1 to about 6 carbon atoms with alkyl branching at the alpha, beta, gamma, and delta carbons of 1 to about 6 carbon atoms; and

(e) R_4 is selected from the group consisting of hydrogen, alkyl of 1 to about 7 carbon atoms optionally substituted with -OH or benzyloxy and alkyl of 1 to about 3 carbon atoms substituted on the terminal carbon atom with aryl of about 4 carbon atoms to about 10 carbon atoms which is optionally mono-, di-, or tri-substituted with Y_1, Y_2 and/or Y_3.

Preferred X groups include $-SO_2-$, $-NH-S(O)_2-$, and $-N(R')-S(O)_2$. Especially preferred X groups include $-SO_2$.

Preferred R_1 groups include alkyl, aralkyl, and aryl groups. Suitable aralkyl and aryl groups include substituted or unsubstituted benzyl and naphthyl, respectively. Preferred substitutions include, $-C(O)OH$, $-C(O)OZ_1$, $-S(O)_mZ_1$, and $-CF_3$. Meta and ortho substitution is preferred. Ortho substitution is particularly preferred. Especially preferred R_1 groups include aralkyl groups. Particularly preferred R_1 groups include substituted or unsubstituted benzyl groups. Cyclohexyl and cyclohexylmethyl also are especially preferred R_1 groups.

Preferred R_2 groups include $-CH_2CH_2CH_2NHC(=NH)NH_2$, $-CH_2CH_2S(O)_2CH_3$, $-CH_2S(O)_2(CH_2)_pC(O)Z_5$, $-(CH_2)_pC(O)NR_5R_6$, $-(CH_2)_pCOOH$, and . Particularly preferred are $-CH_2CH_2CH_2NHC(=NH)NH_2$, $-CH_2CH_2S(O)_2CH_3$, and $-(CH_2)_pC(O)NR_5R_6$. Especially preferred is $-CH_2CH_2CH_2NHC(=NH)NH_2$. Preferred R_5 groups include hydrogen. Preferred R_6 groups include hydrogen, 3-(R)-quinuclidine, 3-(S)-quinuclidine, 4-trifluoromethyl-7-yl-

coumarin, 4-methyl-7-yl-coumarin, 7-yl-coumarin, 3-yl-2-ethyl-4(3H)-quinazolinone, 2-yl-benzothiazole, 3-yl-benzoic acid, 3-yl-4-hydroxybenzoic acid, 4-hydroxy-1-methyl-6-phenyl-3-yl-2(1H)-pyridone, and 1-adamantyl, or ethyl morpholine, ethyl piperidine, 2-(2-ethyl)pyridine, 4-hydroxyphenethyl, (R)-alpha-methylbenzyl, (S)-alpha-methylbenzyl, 4-(methyl)-5-hydroxy-6-methyl-3-pyridine methanol, (1R,2S)-(N-methyl-N-(1-ethyl))benzyl alcohol, (1S,2R)-(N-methyl-N-(1-ethyl))benzyl alcohol, (1R,2R)-(N-methyl-N-(1-ethyl))benzyl alcohol, (1S,2S)-(N-methyl-N-(1-ethyl))benzyl alcohol, and 4-(methyl)-5-hydroxy-6-methyl-3-pyridine methanol..

A preferred group of quaternary ammonium salts on the R_2 group are those alkylated with alkyl of 1 to about 10 carbon atoms. Particularly preferred straight chain quaternary ammonium salts are those with methyl, ethyl, propyl and butyl. Preferred branched alkyl quaternary ammonium salts include isopropyl, isobutyl, isopentyl, and isoamyl. Preferred cyclic quaternary ammonium salts include cyclohexyl, cyclopentyl, and cyclohexylmethyl. Another preferred group of quaternary ammonium salts are those alkylated with aralkyl of 7 to about 15 carbon atoms. Particularly preferred aralkyl groups for quaternary ammonium salts include benzyl and phenethyl. Another preferred group of quaternary ammonium salts are those alkylated with aryl of 6 to about 14 carbon atoms, optionally substituted.

Preferred counterions to the quaternary ammonium salts include chlorine, bromine, iodine, acetate, and trifluoroacetate.

Preferred R_3 groups include alkyl groups of 1 to about 7 carbon atoms optionally substituted with -OH on a terminal carbon atom. Other preferred R_3 groups include methyl, cyclohexylmethyl, phenyl, and benzyl. Particularly preferred R_3 groups are methyl, and cyclohexyl.

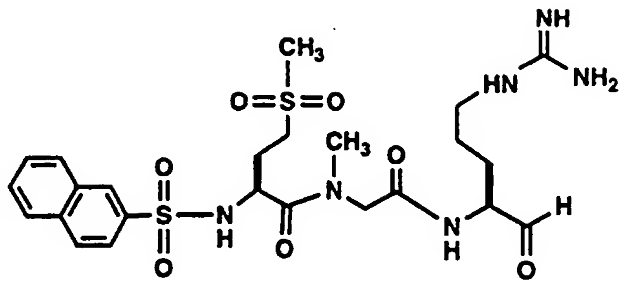
Preferred R_4 groups include hydrogen and alkyl groups of 1 to about 7 carbon atoms optionally substituted with

-OH on a terminal carbon atom. Particularly preferred is hydrogen.

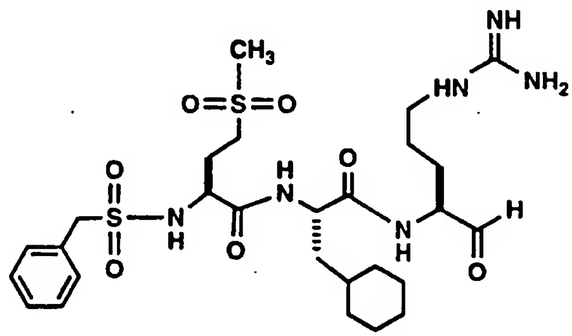
According to a particularly preferred aspect, provided are compounds of formula I wherein X is $-S(O)_2-$, R_1 is substituted or unsubstituted aralkyl, and R_2 is $-CH_2CH_2CH_2NHC(=NH)NH_2$, R_3 is methyl, and R_4 is hydrogen.

Another particularly preferred aspect are compounds of formula I wherein X is $-S(O)_2-$, R_1 is substituted or unsubstituted aralkyl, and R_2 is $-CH_2CH_2CH_2NHC(=NH)NH_2$, R_3 is cyclohexyl, and R_4 is hydrogen. A very preferred aspect is directed to such compounds where R_1 is substituted or unsubstituted benzyl.

Preferred compounds include D-camphorsulfonyl aspartyl sarcosine arginine aldehyde (Example 10), D-camphorsulfonyl cysteinesulfone-acetic acid sarcosine-arginine aldehyde (Example 44), D-camphorsulfonyl-L-methionine sulfone sarcosine-arginine aldehyde (Example 28), benzylsulfonyl-D-arginine-sarcosine-arginine aldehyde (Example 16), (2-carbomethoxy)benzenesulfonyl-(D)-argininyl-sarcosine-argininal (Example 21), N-benzylsulfonyl-(D)-methioninylsulfone sarcosine argininal (Example 35), benzylsulfonylmethioninyl(sulfone)N-cyclohexylglycinylargininal (Example 50), (D)-camphorsulfonyl aspartyl sarcosine arginine aldehyde (Example 56), (D)-camphorsulfonyl-3-(3-pyridyl)-alanine sarcosine arginine aldehyde (Example 62), benzylsulfonyl-3-(3-pyridyl)-alanine sarcosine arginine aldehyde (Example 67), (D)-camphorsulfonyl-glycine-sarcosine-arginine aldehyde (Example 73), d-camphor sulfonyl-glutamyl(O-methyl)-sarcosyl-argininal, benzylsulfonyl-glutamyl(O-methyl)-sarcosyl-argininal, d-camphorsulfonyl-glutaminyl-sarcosyl-argininal, d-camphorsulfonyl-glutamyl-sarcosyl argininal,



(a),



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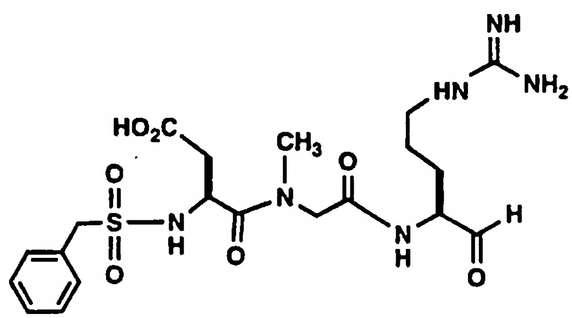

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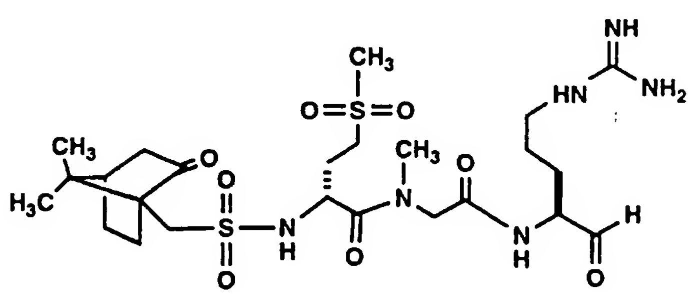

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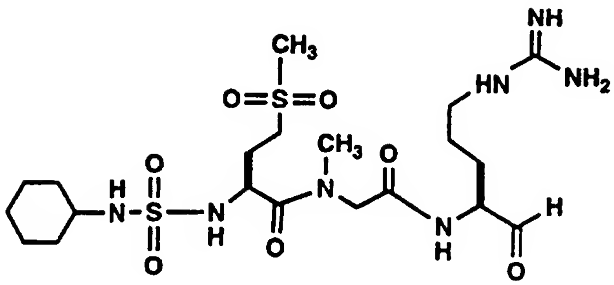
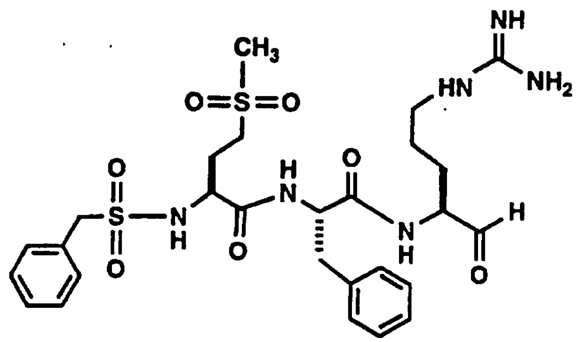
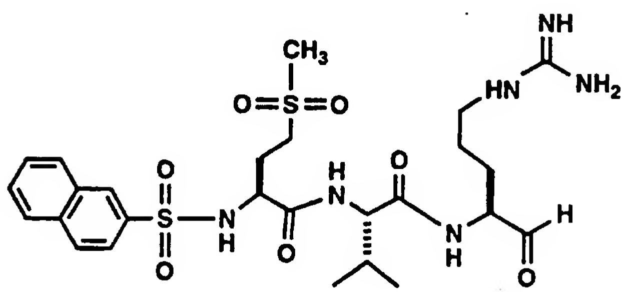


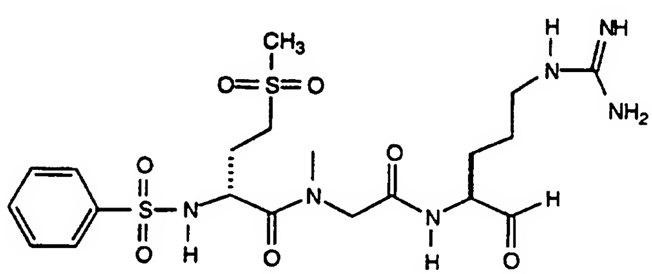
(e),



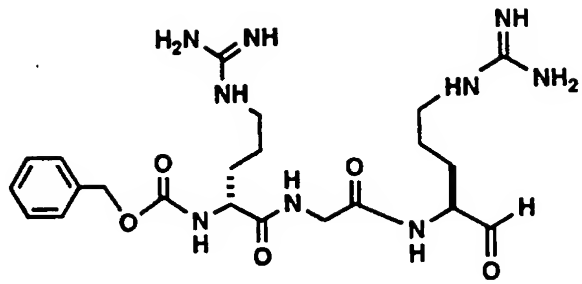
(f),



(g),



(h), and



(i).

Thus, preferred compounds of formula (I) also include the compounds of the following Examples: 56 and 115A, 16 and 116A, 116C, 117, 115C, 118E, 115D, 116F, 108 and 120E, 114 and 120H, 113 and 120G, and 120J.

According to another aspect, the present invention is directed to salts of the compounds of formula (I). "Salt" includes within its definition, salts of the compounds of the present invention derived from the combination of such compounds and an organic or inorganic acid. In practice, the use of the salt form amounts to use of the base form. The compounds of the present invention are useful in both free base and salt form, with both forms being considered as being within the scope of the present invention. These salts include acid addition salts, for example, salts of hydrochloric acid, hydrobromic acid, acetic acid, benzene sulfonic acid and other suitable acid addition salts. These salts include salts formed from compounds containing quaternary ammonium salts.

2. Preparation of Preferred Compounds

The compounds of the present invention may be prepared by the preferred reaction schemes depicted in Figures 1, 3, 4, and 5. Examples 1 through 4 provide the details of a preferred method of making the commonly utilized intermediate, N^g-nitro-L-argininal ethyl cyclol.

As shown in Figure 1, various Boc protected amino acids are coupled to sarcosine benzyl ester. Other N-alkylated amino acids may be used. The Boc group is removed with hydrochloric acid. The hydrochloride salt of the terminal free amine is reacted with triethylamine, and

R_1SO_2Cl in acetonitrile to give the sulfonamide, which is then hydrogenat d with hydrogen gas and palladium on carbon in a Parr Shaker to remove the benzyl ester protecting group. The free acid is coupled to N^g-nitro-L-argininal ethyl cyclol hydrochloride salt (prepared as described in Examples 1 through 4) by carbodiimide coupling.

The N^g-nitro group of the adduct is then removed by hydrogenation with hydrogen gas and palladium on carbon in ethanol, water, and acetic acid. Then the compound is reacted with 3N hydrochloric acid or hexafluorophosphoric acid to provide the argininal compound of the invention.

Preferred means of chemically coupling (as for example, the first step in Figure 1) include formation of a peptide bond by using conventional coupling reagents known in the art. See Bodanszky, N., Peptide Chemistry, pp. 55-73, Springer-Verlag, New York (1988) and references cited therein. The chemical coupling may be either by means of one-step or two-step coupling. In one-step coupling, the two coupling partners are coupled directly. Preferred coupling reagents for one-step coupling include N,N'-dicyclohexylcarbodiimide with 1-hydroxybenzotriazole monohydrate, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide with 1-hydroxybenzotriazole monohydrate, benzotriazol-1-yl-oxy-tris(dimethylamino)phosphonium hexafluorophosphate, 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate or 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate. In two-step coupling, an activated ester or anhydride of the C-terminal carboxy group of one coupling partner is formed prior to its coupling to the other coupling partner.

For compounds of the present invention containing alkenyl or aryl moieties substituted with halogen, cyano, nitro, or -S-Z_1, it is preferred to avoid the use of hydrogen gas with palladium on carbon. Instead, it is preferred to use boron tris(trifluoroacetate), $B(OCOCF_3)_3$, to cleave the N^g-nitro protecting the arginine group. The reagent is prepared by the reaction of BBr_3 and CF_3COOH in

dichloromethane at 0°C. The reagent is also commercially available. Generally, the N^g-nitro compound is treated with boron tris(trifluoroacetate) in trifluoroacetic acid at 0°C. Fieser, M. and Fieser, L. F., Reagents for Organic Synthesis, p. 46, John Wiley & Sons, New York (1974); Pless, J., and Bauer, W. (1973) Angew. Chem., Internat. Ed., 12, 147.

An even more preferred method is to use the di-N-t-butoxycarbonyl protecting group for the L-argininal moiety for groups incompatible with hydrogenation with palladium on carbon. For example, alpha-N-t-benzyloxycarbonyl-omega, omega'-di-N-t-butoxycarbonylarginine is dissolved in acetonitrile and treated with hydroxybenzotriazole and 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloric acid salt to form alpha-N-benzyloxycarbonyl-omega, omega'-di-N-t-butoxycarbonyl-L-arginine lactam. The lactam is opened by treatment with $LiAlH_4$ in tetrahydrofuran at -70°C to provide alpha-N-benzyloxycarbonyl-omega, omega'-di-N-t-butoxycarbonyl-L-argininal. This aldehyde is protected as the diethyl acetal by treatment with ethanol and hydrochloric acid. The N-benzyloxycarbonyl protecting group is removed by treatment with hydrogen gas and palladium on carbon to give omega, omega'-di-N-t-butoxycarbonyl-L-argininal diethyl acetal, hydrochloric acid salt. This protected L-argininal moiety can then be coupled to a desired carboxylic acid by treatment with N-hydroxybenzotriazole and 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloric acid salt. The diethyl acetal and the di-BOC protecting groups are removed by treatment with hexafluorophosphonic acid in acetonitrile at 0°C. The reaction is quenched with 2.5 M aqueous sodium acetate until pH 4 is reached. The mixture is filtered through a 2 micron filter. Preparative HPLC using 0.1% CF_3COOH in 10-40% aqueous acetonitrile provides the trifluoroacetate salt of the desired substituted L-argininal compound.

In Figure 3, the Boc protected methionine sulfone sarcosine benzyl ester, prepared in Example 74a, is N-

alkylated with (2-iodoethyl) benzene. Various N-alkylating agents can be used. The benzyl ester protecting group is removed by treatment with hydrogen gas and palladium on carbon. The resulting free acid is coupled to the compound of Example 4 by treatment with 1-ethyl-3(-dimethylaminopropyl)carbodiimide, N-hydroxybenzotriazole, and N-methyl morpholine. The N^g-nitro group is removed by treatment with hydrogen gas, palladium on carbon in methanol, acetic acid and water mixture. The cyclic arginine ethylaminal is opened by treatment with hexafluorophosphoric acid in water to give the final product. Figure 3 depicts a scheme for the synthesis of compounds where X is a direct link. Examples 74a through 78 describe such a synthesis.

Figure 4 illustrates a preferred method of synthesizing compounds with an N-alkylated R_2 group. "N-alkylated" encompasses N-alkylated, N-arylated, and N-aralkylated quaternary ammonium salts. Examples 79 through 89 provide the details of this synthetic scheme.

The Boc protected glumatic acid benzyl ester is coupled to 3-(R)-aminoquinuclidine dihydrochloride using standard procedures. Various amines can be used. Strong acid removes the Boc protecting group. The amine is then reacted with triethylamine and benzylsulfonyl chloride. The benzyl ester protecting group is removed by catalytic hydrogenation. The free acid is then coupled to the compound of Example 4 using standard coupling reagents. The basic amine of the R_2 group is then reacted with allyliodide. The N^g-nitro group is removed and the allyl group reduced by treatment with hydrogen gas and palladium on carbon. The cyclic arginine ethylaminal is opened with hexafluorophosphoric acid or 6 M hydrochloric acid in water to give the final product.

Figure 5 illustrates a preferred reaction scheme for the preparation of N-benzylsulfonyl-L-cysteine sulfone-S-((R)-alphamethylbenzylcarboxyamide)-sarcosine argininal. Examples 90 through 96 describe the reaction scheme. The Boc group of butyl acetate cysteinesulfone sarcosine-O-

benzyl ester, prepared according to Examples 36 through 39 is removed by treatment with anhydrous hydrochloric acid. The terminal amine is reacted with an amine base and R_1SO_2Cl to provide the sulfonamide. The t-butyl ester is reacted with trifluoroacetic acid to form the carboxylic acid. The acid is then coupled to (R)-alphamethyl benzylamine using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt and 1-hydroxybenzotriazole. The resulting adduct is then treated with hydrogen gas and palladium on carbon to remove the benzyl ester protecting group. The resulting acid is coupled to the compound of Example 4 using standard coupling techniques. The N^g-nitro group is removed with catalytic hydrogenation and then the argininal ethyl cyclol aminal is opened with hexafluorophosphoric acid or 6 M hydrochloric acid in water to give the argininal product.

3. Selection of Preferred Compounds

The compounds of the present invention are screened for their ability to inhibit thrombin, plasmin, recombinant tissue plasminogen activator (rt-PA), activated protein C (aPC), chymotrypsin, and trypsin as set forth below. Certain of the preferred compounds are distinguished by their ability to inhibit factor Xa, while not substantially inhibiting thrombin, plasmin, t-PA, aPC, chymotrypsin, and trypsin. With respect to factor Xa and the other enzymes and as used herein, the term "not substantially inhibiting" means that the IC_{50} (or K_i) for thrombin, plasmin, t-PA, aPC, chymotrypsin, and trypsin for a given compound is greater than or equal to its IC_{50} (or K_i, respectively) for factor Xa.

The compounds of the present invention are dissolved in buffer to give solutions containing concentrations such that assay concentrations range from 0 to 100 micromolar. In the assays for factor Xa, thrombin, plasmin, t-PA, aPC, chymotrypsin, and trypsin, a chromogenic synthetic substrate is added to a solution containing test compound

and the enzyme of interest and the residual catalytic activity of that enzyme is determined spectrophotometrically. The IC_{50} of a compound of the present invention is determined from the rate of substrate turnover caused by the specific enzyme being measured. IC_{50} is that concentration of test compound giving 50% inhibition of the rate of substrate turnover. Likewise, the K_i of a compound of the present invention is determined from the rate of substrate turnover caused by the specific enzyme being measured at various enzyme concentrations. K_i is that concentration of test compound giving 50% inhibition of the rate of substrate turnover. Examples A and B provide an exemplar of the in vitro assays used to select the compounds of the present invention.

Certain of the preferred compounds of the present invention have a K_i of about 0.001 to about 200 nM in the factor Xa assay. Especially preferred compounds have a K_i of about 0.001 to about 50 nM. The more especially preferred compounds have a K_i of about 0.001 to about 10 nM.

Certain of the preferred compounds of the present invention have a IC_{50} for thrombin, plasmin, t-PA, aPC, chymotrypsin, and trypsin which is at least 10 times greater than its IC_{50} for factor Xa. Especially preferred compounds have an IC_{50} for thrombin, plasmin, rt-PA, aPC, chymotrypsin, and trypsin which is about 20 to about 100,000 times greater than its IC_{50} for thrombin. More especially preferred compounds have an IC_{50} for thrombin, plasmin, rt-PA, aPC, chymotrypsin, and trypsin which is about 100 to about 1,000,000 times greater than its IC_{50} for factor Xa. In the event that a compound of the present invention has an IC_{50} with respect to thrombin, plasmin, rt-PA, aPC, chymotrypsin, or trypsin which is greater than the highest concentration of compound tested, the IC_{50} is taken to be that highest concentration of compound.

4. Pharmaceutical Compositions

In another aspect, the present invention encompasses pharmaceutical compositions prepared for storage or administration which comprise a therapeutically effective amount of a compound of the present invention in a pharmaceutically acceptable carrier.

The therapeutically effective amount of a compound of the present invention will depend on the route of administration, the type of mammal being treated, and the physical characteristics of the specific mammal under consideration. These factors and their relationship to determining this amount are well known to skilled practitioners in the medical arts. This amount and the method of administration can be tailored to achieve optimal efficacy but will depend on such factors as weight, diet, concurrent medication and other factors which those skilled in the medical arts will recognize.

The therapeutically effective amount of a compound of the present invention can range broadly depending upon the desired effects and the therapeutic indication. Typically, dosages will be between about 0.01 mg/kg and 100 mg/kg body weight, preferably between about 0.01 and 10 mg/kg, body weight.

Pharmaceutically acceptable carriers for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co. (A.R. Gennaro edit. 1985). For example, sterile saline and phosphate-buffered saline at physiological pH may be used. Preservatives, stabilizers, dyes and even flavoring agents may be provided in the pharmaceutical composition. For example, sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid may be added as preservatives. Id. at 1449. In addition, antioxidants and suspending agents may be used. Id.

The pharmaceutical compositions of the present invention may be formulated and used as tablets, capsules or elixirs for oral administration; suppositories for

rectal administration; sterile solutions and suspensions for injectable administration; and the like. The dose and method of administration can be tailored to achieve optimal efficacy but will depend on such factors as weight, diet, concurrent medication and other factors which those skilled in the medical arts will recognize.

When administration is to be parenteral, such as intravenous on a daily basis, injectable pharmaceutical compositions can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Suitable excipients are, for example, water, saline, dextrose, mannitol, lactose, lecithin, albumin, sodium glutamate, cysteine hydrochloride, or the like. In addition, if desired, the injectable pharmaceutical compositions may contain minor amounts of nontoxic auxiliary substances, such as wetting agents, pH buffering agents, and the like. If desired, absorption enhancing preparations (e.g., liposomes) may be utilized.

5. Utility and Methods

Compounds of the present invention when made and selected as disclosed are useful as potent inhibitors of factor Xa in vitro and in vivo. As such, these compounds are useful as in vitro diagnostic reagents to prevent the clotting of blood and as in vivo pharmaceutical agents to prevent thrombosis in mammals suspected of having a condition characterized by abnormal thrombosis.

The compounds of the present invention are useful as in vitro diagnostic reagents for inhibiting clotting in blood drawing tubes. The use of stoppered test tubes having a vacuum therein as a means to draw blood obtained by venipuncture into the tube is well known in the medical arts. Kasten, B.L., "Specimen Collection", Laboratory Test Handbook, 2nd Edition, Lexi-Comp Inc., Cleveland pp. 16-17 (Edits. Jacobs, D.S. et al. 1990). Such vacuum tubes may be free of clot-inhibiting additives, in which case, they are useful for the isolation of mammalian serum from the

blood. They may alternatively contain clot-inhibiting additives (such as heparin salts, EDTA salts, citrate salts or oxalate salts), in which case, they are useful for the isolation of mammalian plasma from the blood. The compounds of the present invention are potent inhibitors of factor Xa, and as such, can be incorporated into blood collection tubes to prevent clotting of the mammalian blood drawn into them.

The compounds of the present invention are used alone, in combination of other compounds of the present invention, or in combination with other known inhibitors of clotting, in the blood collection tubes. The amount to be added to such tubes is that amount sufficient to inhibit the formation of a clot when mammalian blood is drawn into the tube. The addition of the compounds to such tubes may be accomplished by methods well known in the art, such as by introduction of a liquid composition thereof, as a solid composition thereof, or liquid composition which is lyophilized to a solid. The compounds of the present invention are added to blood collection tubes in such amounts that, when combined with 2 to 10 mL of mammalian blood, the concentration of such compounds will be sufficient to inhibit clot formation. Typically, the required concentration will be about 1 to 10,000 nM, with 10 to 1000 nM being preferred.

The compounds of the present invention are useful as pharmaceutical agents for preventing thrombosis in a mammal suspected of having a condition characterized by abnormal thrombosis.

Conditions characterized by abnormal thrombosis are well known in the medical arts and include those involving the arterial and venous vasculature of mammals. With respect to the coronary arterial vasculature, abnormal thrombosis (thrombus formation) characterizes the rupture of an established atherosclerotic plaque which is the major cause of acute myocardial infarction and unstable angina, as well as also characterizing the occlusive coronary thrombus formation resulting from either

thrombolytic therapy or percutaneous transluminal coronary angioplasty (PTCA). With respect to the venous vasculature, abnormal thrombosis characterizes the condition observed in patients undergoing major surgery in the lower extremities or the abdominal area who often suffer from thrombus formation in the venous vasculature resulting in reduced blood flow to the affected extremity and a predisposition to pulmonary embolism. Abnormal thrombosis further characterizes disseminated intravascular coagulopathy which commonly occurs within both vascular systems during septic shock, certain viral infections and cancer, a condition wherein there is rapid consumption of coagulation factors and systemic coagulation which results in the formation of life-threatening thrombi occurring throughout the microvasculature leading to widespread organ failure.

The present invention includes methods for preventing a condition in a mammal suspected of having a condition characterized by abnormal thrombosis, comprising administering to said mammal a therapeutically effective amount of a compound or a pharmaceutical composition of the present invention.

The compounds or pharmaceutical compositions of the present invention are administered in vivo, ordinarily in a mammal, preferably in a human. In employing them in vivo, the compounds or pharmaceutical compositions can be administered to a mammal in a variety of ways, including orally, parenterally, intravenously, subcutaneously, intramuscularly, colonically, rectally, nasally or intraperitoneally, employing a variety of dosage forms. Administration is preferably parenteral, such as intravenous on a daily basis. Alternatively, administration is preferably oral, such as by tablets capsules or elixirs taken on a daily basis.

In practicing the methods of the present invention, the compounds or pharmaceutical compositions of the present invention are administered alone or in combination with one another, or in combination with other therapeutic

or in vivo diagnostic agents.

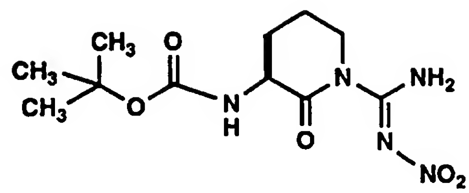
As is apparent to one skilled in the medical art, a therapeutically effective amount of the compounds or pharmaceutical compositions of the present invention will vary depending upon the age, weight and mammalian species treated, the particular compounds employed, the particular mode of administration and the desired effects and the therapeutic indication. Because these factors and their relationship to determining this amount are well known in the medical arts, the determination of therapeutically effective dosage levels, the amount necessary to achieve the desired result of preventing thrombosis, will be within the ambit of one skilled in these arts. Typically, administration of the compounds or pharmaceutical compositions of the present invention is commenced at lower dosage levels, with dosage levels being increased until the desired effect of preventing in vivo thrombosis is achieved which would define a therapeutically effective amount. For the compounds of the present invention, alone or as part of a pharmaceutical composition, such doses are between about 0.01 mg/kg and 100 mg/kg body weight, preferably between about 0.01 and 10 mg/kg, body weight.

To assist in understanding, the present invention will now be further illustrated by the following examples. These examples as they relate to this invention should not, of course, be construed as specifically limiting the invention and such variations of the invention, now known or later developed, which would be within the purview of one skilled in the art are considered to fall within the scope of the invention as described herein and hereinafter claimed.

Examples

Example 1

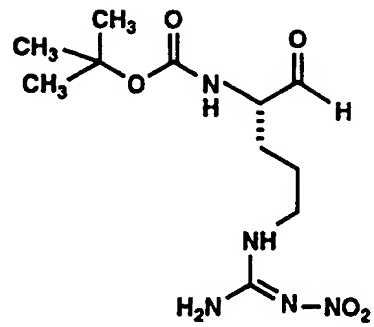
Preparation of N-alpha-t-butoxycarbonyl-N^g-nitro-L-arginine lactam



N-alpha-t-butoxycarbonyl-N^g-nitroarginine (2.00 g, 6.3 mmole) was dissolved in tetrahydrofuran (100 mL) by heating the solution to 50°C. The solution was allowed to cool to room temperature. N-methylpiperidine (0.84 mL, 6.9 mmole) was added, and the solution was cooled in an ice bath. Isobutylchloroformate (0.83 mL, 6.3 mmole) was added, and the reaction mixture was stirred at 0°C for 6 hours. The reaction mixture was stirred for 18 hours while the ice in the dewar was allowed to melt overnight. The solvent was removed under vacuum. The crude product was dissolved in 20% ethyl acetate/dichloromethane (10 mL), and was purified by flash chromatography through a 3x5 cm column of silica gel using 20% ethyl acetate/dichloromethane as eluent. 125 mL of eluent was collected. The solvent was removed under vacuum to afford 1.39 g (74% crude yield) of the title compound as a white foam. R_f = 0.44 (silica gel, 95:5, dichloromethane:isopropanol). Isobutanol was present as an impurity. This compound may be further purified by recrystallization from dichloromethane/hexanes or ethanol/water.

Example 2

Preparation of N-alpha-t-butoxycarbonyl-N^g-nitro-L-argininal



(a) Procedure 1

To a stirred solution of $LiAlH_4$ in tetrahydrofuran (3.8 mL of a 1.0M solution, 3.8 mmole), cooled in an ice bath, was added dropwise ethyl acetate (0.43 mL, 3.8 mmole) in tetrahydrofuran (5 mL). The solution was stirred for 30 minutes at 0°C to preform $LiAlH_2(OEt)_2$.

The solution of this $LiAlH_2(OEt)_2$ was added dropwise to a stirred solution of compound of Example 1 (0.92 g, 3.1 mmole) in tetrahydrofuran (5 mL). After 30 minutes, the reaction is quenched with 1.0N aqueous hydrochloric acid/tetrahydrofuran (2 mL of a 1:1 mixture). 1.0N aqueous hydrochloric acid (20 mL) was added, and the solution was extracted three times with ethyl acetate (20 mL each). The combined organic layers were washed with water (5 mL), saturated sodium bicarbonate (5 mL) and twice with brine (2x5 mL), dried over anhydrous magnesium sulfate, filtered and the solvent was removed under vacuum to give 0.94 g (100% yield) of the title compound as an off-white solid.

(b) Procedure 2

Alternatively, the title compound was made by the procedures which follow.

A 12 liter four-necked round bottom flask equipped with an overhead stirring apparatus was flame dried under a strong stream of nitrogen. After the flask had cooled, 120.0 g of N-alpha-t-butoxycarbonyl-N^g-nitro-L-arginine (376 mmole, 1 equivalent) was added under a blanket of nitrogen followed by the addition of 6 liters of anhydrous

tetrahydrofuran (Aldrich sure-seal) via cannula. The flask was then fitted with a thermometer and the resulting suspension was warmed to 50°C with a heat gun while stirring. The reaction mixture was cooled to 5°C with an ice bath and further cooled to -5°C with an ice/acetone bath.

During the time it took for this solution to reach -5°C, 36.66 g of N,O-dimethylhydroxylamine hydrochloride (376 mmole, 1.0 equivalent) was weighed out in a 500 mL flask and suspended in 300 mL of dichloromethane. This suspension was sparged with nitrogen for 5 minutes, cooled to 0°C and 46 mL of N-methylpiperidine (1.0 equivalent) was added via syringe under nitrogen. The mixture was sonicated briefly to insure complete dissolution/free base formation and recooled to 0°C in an ice bath while still under nitrogen. The resulting solution of free base was used later.

When the above arginine solution had reached -5°C, 45 mL of N-methylpiperidine was added via syringe followed 5 minutes later by the addition of 46 mL of isobutyl chloroformate (0.95 equivalent) via syringe. The resulting solution was stirred for 15 minutes at -5°C. After this time, the free base solution of N,O-dimethylhydroxylamine generated above was added via cannula over about 15 minutes. Stirring was continued at -5°C for another 1.5 hours at which time thin layer chromatography (silica gel, 1:10:90 acetic acid / methanol / dichloromethane) indicated that the reaction was complete. The reaction mixture was filtered while still cold, the salts washed with 400 mL of cold tetrahydrofuran and the filtrate concentrated under vacuum on a rotary evaporator to yield a yellow foam.

The crude intermediate was taken up in 300 mL of dichloromethane and applied to a column of silica gel (70-230 mesh, 7x50 cm). The column was first eluted with 2 liters of dichloromethane followed by 2 liters of 2% methanol in dichloromethane. This was followed by elution

with 5% methanol in dichloromethane until all of the product had been eluted (the eluent was checked for UV activity and five one-liter fractions were collected once this UV activity was apparent). Fractions containing pure product were pooled and concentrated under vacuum and placed under a high vacuum overnight to yield 120.1 g (88% yield) of N-alpha-t-butoxycarbonyl-N^g-nitro-L-arginine-(N,O-dimethylhydroxylamide) as a light yellow foam. This foam was taken up in 300 mL of dichloromethane, 300 mL of toluene, and the volatiles were once again removed under vacuum to remove any residual water or methanol.

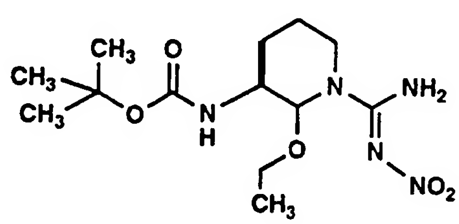
120.1 g of N-alpha-t-butoxycarbonyl-N^g-nitro-L-arginine-(N,O-dimethylhydroxylamide) (331.4 mmole) was taken up in 2.8 liters of dry (Aldrich sure-seal) tetrahydrofuran and transfered to a dry 5 liter 4-necked round bottom flask equipped with a mechanical stirrer and a low temperature thermometer. The solution was cooled to -70°C with a dry ice/acetone bath and 300 mL of 1M $LiAlH_4$ in tetrahydrofuran was added by cannula transfer directly from 100 mL Aldrich sure-seal bottles. An additional 50 mL of 1M $LiAlH_4$ in tetrahydrofuran was added via syringe (total 331 mL). During the additions, the reaction temperature was kept below -60°C. The reaction was stirred for 0.5 hours at -70°C, the cooling bath removed, and the reaction was slowly allowed to warm to 0°C (about 2.5 hours). Between -30°C and -20°C a thick slurry resulted. When the reaction mixture obtained 0°C, a small aliquot was removed and partitioned between ethyl acetate/2M potassium bisulfate. The organic layer was then analyzed by thin layer chromatography (silica gel, ethyl acetate).

When the reaction was judged to be complete, it was cooled to -70°C and 503 mL of 2M potassium bisulfate was added via dropping funnel at a slow enough rate to keep the reaction temperature below -30°C. The cooling bath was removed and the reaction mixture was allowed to come

to 0°C over the course of 2 hours at which time a white precipitate was filtered off. The solids were washed with 500 mL of cold tetrahydrofuran. The filtrate was concentrated under vacuum on a rotary evaporator until most of the tetrahydrofuran was removed and the remaining white sludge was mostly aqueous. The crude product was taken up in 1.5 liters of ethyl acetate and washed with 0.2 M hydrochloric acid (2 x 200 mL). The hydrochloric acid extracts were back-extracted with 400 mL of ethyl acetate and the organics were combined and extracted with saturated sodium bicarbonate (2 x 200 mL). The bicarbonate extracts were also back-extracted with 400 mL of ethyl acetate. The organics were then combined and washed with brine (200 mL) followed by drying over anhydrous sodium sulfate. The solution was filtered, concentrated under vacuum on a rotary evaporator and placed on a high vacuum overnight to yield a white solid (89.0 g) of crude title compound. This was chromatographed on silica gel and eluted with a gradient of 0 to 10% methanol in dichloromethane. The pure fractions were combined and evaporated to yield the title compound as a white solid (75 g, 74%).

Example 3

Preparation of N-alpha-t-butoxycarbonyl-N^g-nitro-L-argininal ethyl cyclol

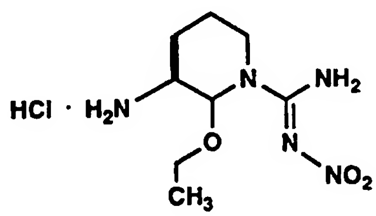


The compound of Example 2 (41.60 g, 0.137 mole) was dissolved in ethanol (200 mL) and concentrated hydrochloric acid (1 mL) was added. After the reaction was complete by TLC (silica gel, 10% methanol in dichloromethane), the solvent was removed under vacuum.

The crude product was purified by flash chromatography through a column of silica gel (230-400 mesh) using 0 to 10% ethyl acetate/dichloromethane as eluent. The combined fractions yielded 36.88 g (81%) of the title compound as pale yellow foam. R_f = 0.62 (silica gel, 95:5, CH_2Cl_2:methanol).

Example 4

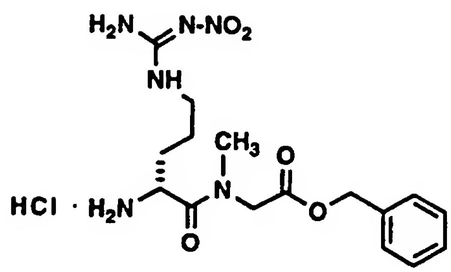
Preparation of N^g-nitro-L-argininal ethyl cyclol, hydrochloride salt



To a solution of the compound of Example 3 (35 g) in 500 mL of absolute ethanol at 0°C was added slowly 500 mL of absolute ethanol saturated with hydrochloric acid (g). This mixture was allowed to warm to 25°C and checked by thin-layer chromatography. The appearance of a very polar product was the desired compound. Most of the hydrochloric acid was removed with a stream of dry nitrogen and the resulting organic solvent was removed under vacuum. The resulting 33 g of the title compound as a yellow-white solid was used without further purification.

Example 5

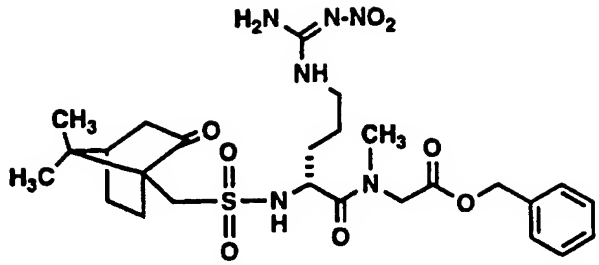
Preparation of (D)-N^g-NO_2-arginine sarcosine benzyl ester hydrochloride salt



To a stirring solution of Boc-D-N^g-nitroarginine (6.2 g, 19.4 mmole) in 100 mL of dry dimethylformamide was added sarcosine benzyl ester para-toluenesulfonic acid salt (8.2 g, 23.3 mmole) followed by benzotriazol-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (8.6 g, 19.4 mmole) and N-methylmorpholine (10.6 mL, 97.1 mmole). The mixture was stirred for 16 hours at room temperature. The reaction mixture was dissolved in 900 mL of ethyl acetate and washed with 300 mL each of water, 1 M aqueous hydrochloric acid, water, saturated aqueous sodium bicarbonate and brine. The organic phase was dried over anhydrous magnesium sulfate, filtered and the solvent removed *in vacuo*. Rf = 0.39 (1:9 methanol:dichloromethane). The resulting oil was dissolved in dichloromethane (50 mL) and treated with 50 mL of 4.0M solution of hydrochloric acid in dioxane. After 5 hours, the title compound precipitated when the reaction mixture was poured into ether (500 mL) with vigorous stirring. The precipitate was filtered and dried in vacuo to provide 8 g (quantitative yield) of the title compound as an off-white powder.

Example 6

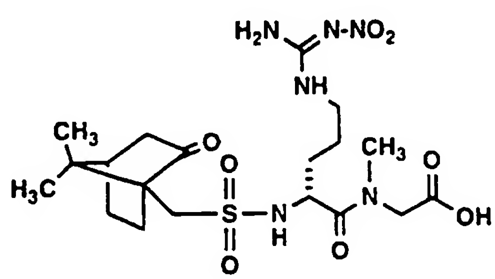
Preparation of (D)-camphorsulfonyl-(D)-N^g-NO_2-arginine sarcosine benzyl ester



To a solution of the compound of Example 5 (4.19 g, 10.1 mmole) in 10 mL of dry dimethylformamide and 50 mL tetrahydrofuran was added (D)-camphorsulfonyl chloride (3.7 g, 15.1 mmole) followed by triethylamine (7.0 mL, 50.3 mmole). The mixture was stirred for 16 hours at room temperature. The reaction mixture was dissolved in 800 mL of ethyl acetate and washed with 200 mL each of water, 1 M aqueous hydrochloric acid, water, saturated aqueous sodium bicarbonate and brine. The organic phase was dried over anhydrous magnesium sulfate, filtered and the solvent removed *in vacuo*. The resulting oil was dissolved in dichloromethane and filtered through silica, eluting first with dichloromethane (500 mL), then 1:9 methanol:dichloromethane (1000 mL). The methanol:dichloromethane fraction was concentrated to provide 5.7 g (95% yield) of the title compound as an off-white foam. Rf = 0.45 (1:9 methanol: dichloromethane).

Example 7

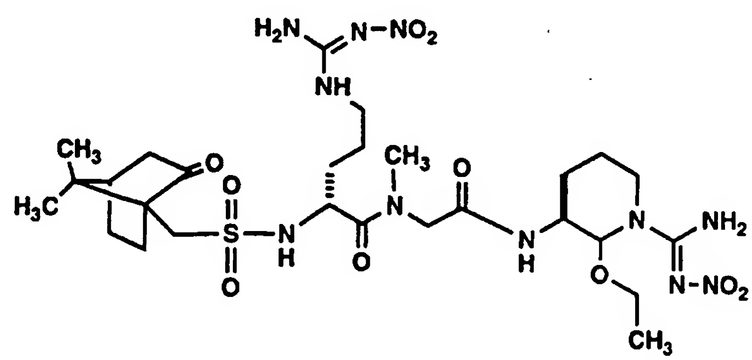
Preparation of (D)-camphorsulfonyl-(D)-N^g-NO_2-arginine sarcosine



10% palladium on carbon (2.5 g) was added to a solution of the compound of Example 6 (5.7 g, 9.57 mmole) in 300 mL of methanol under a nitrogen blanket. The mixture was hydrogenated at 1 atmosphere for 16 hours. The mixture was filtered and then concentrated to provide 4.5 g (96% yield) of the title compound as a white foam.

Example 8

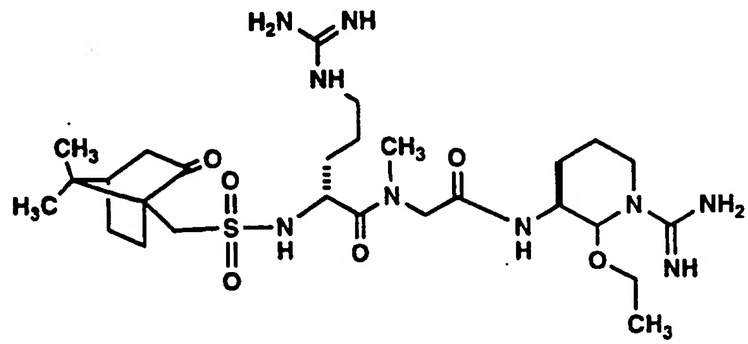
Preparation of (D)-camphorsulfonyl-(D)-N^g-NO_2-arginine-sarcosine-N^g-NO_2-arginine cyclic-OEt aminal



The compound of Example 4 (1.64 g, 6.1 mmole) and the the compound of Example 7 (2 g, 4.1 mmole) were dissolved with stirring in 20 mL of dry acetonitrile. To this mixture was added 1-hydroxy-7-azabenzotriazole (0.28 g, 2.0 mmole) and O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (1.5 g, 4.1 mmole) followed by N-methylmorpholine (1.7 mL, 15.8 mmole). After 16 hours, the reaction mixture was diluted with 600 mL ethyl acetate and extracted with 150 mL each of water, 1 M aqueous hydrochloric acid, water, saturated aqueous sodium bicarbonate and brine. The organic phase was dried over anhydrous magnesium sulfate, filtered and the solvent removed in vacuo. Chromatography of the resulting oil (silica, 4:1:4 hexanes:methanol:dichloromethane) afforded 1.25 g (43% yield) of the title compound as an off-white foam. Rf = 0.26 (1:9 methanol:dichloromethane).

Example 9

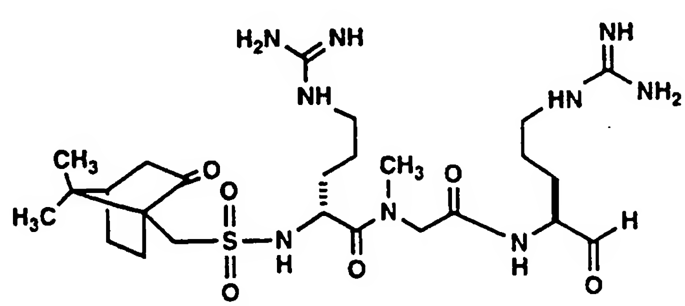
Preparation of (D)-camphorsulfonyl-(D)-arginine-sarcosine-arginine cyclic-OEt aminal



1.25 g of 10% palladium on carbon was placed in a 500 mL PARR bottle to which 10 mL of water and 4 mL of glacial acetic acid was added. To this mixture was added a solution of the compound of Example 8 (1.25 g, 1.74 mmole) in 100 mL of methanol. The mixture was then shaken under a hydrogen atmosphere at 40 psi for 3 days. The catalyst was removed by filtration and the filtrate concentrated in vacuo. The resulting oil was azeotroped with toluene to remove the remaining acetic acid to afford about 1 g (quantitative yield) of the title compound.

Example 10

Preparation of (D)-camphorsulfonyl-(D)-arginine-sarcosine-arginine aldehyde

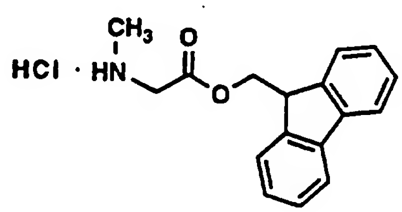


The compound of Example 9 (1 g, 1.7 mmole) was dissolved in 20 mL of 50:50 water:acetonitrile with

stirring and cooled to 0°C in an ice water bath. To this solution was slowly added 50 mL of a 60 wt% solution of hexafluorophosphoric acid in water. After 1 hour, the pH of the reaction mixture was adjusted to about pH 4 using saturated aqueous sodium acetate. This mixture was filtered through a plug of Celite. The title compound was obtained by purification of the filtrate by preparative HPLC (2 inch Vydak C18 column using a gradient consisting of 7 to 28% acetonitrile in water containing 0.1% trifluoroacetic acid run over 60 minutes at a flowrate of 115 mL/minute) and lyophilization of the pooled fractions. Mass Spec(FAB) confirmed the calculated molecular weight of 598.7.

Example 11

Preparation of sarcosine-O-fluorenylmethyl ester hydrochloride salt

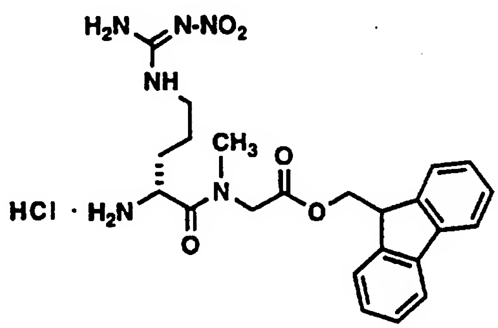


To a solution of Boc-sarcosine (30 g, 158 mmole) in 500 mL dichloromethane was added with stirring carbonyl diimidazole (25.7 g, 158 mmole). After 15 minutes, 9-fluorene methanol (29.5 g, 150 mmole) was added and stirring was continued. After 16 hours, the reaction mixture was diluted with 1200 mL ethyl acetate and extracted with 300 mL each of water, 1 M aqueous hydrochloric acid, water, saturated aqueous sodium bicarbonate and brine. The organic phase was dried over anhydrous magnesium sulfate, filtered and the solvent removed *in vacuo*. The resulting oil was dissolved in 500 mL dichloromethane and treated with 100 mL of a 4.0M solution of hydrochloric acid in dioxane. After 12 hours, the title compound was precipitated by pouring the

reaction mixture into ether (1000 mL) with vigorous stirring. The precipitate was filtered and dried in vacuo to provide 35 g (73% yield) of the title compound as an off-white powder.

Example 12

Preparation of (D)-N^g-NO_2-arginine sarcosine-O-fluorenylmethyl ester hydrochloride salt



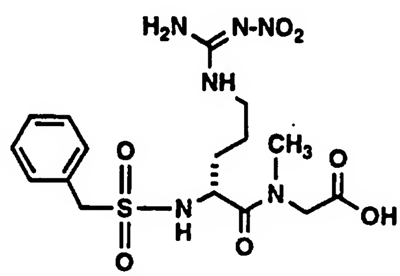
To a stirring solution of Boc-(D)-N^g-nitroarginine (10 g, 31.3 mmole) in 150 mL of dry dimethylformamide was added the compound of Example 11 (21.2 g, 40.7 mmole) followed by benzotriazol-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (13.9 g, 31.3 mmole) and 2,4,6-collidine (120.7 mL, 156.5 mmole). The mixture was stirred for 16 hours at room temperature. The reaction mixture was dissolved in 1200 mL of ethyl acetate and washed with 350 mL each of water, 1 M aqueous hydrochloric acid, water, saturated aqueous sodium bicarbonate and brine. The organic phase was dried over anhydrous magnesium sulfate, filtered and the solvent removed in vacuo. Rf = 0.45 (1:9 methanol:dichloromethane).

The resulting oil was dissolved in dichloromethane (500 mL) and treated with 100 mL of 4.0M solution of hydrochloric acid in dioxane. After 12 hours, the title compound was precipitated by pouring the reaction mixture into diethyl ether (500 mL) with vigorous stirring. The precipitate was filtered and dried in vacuo to provide 15

g (95% yield) of the title compound as an off-white powder.

Example 13

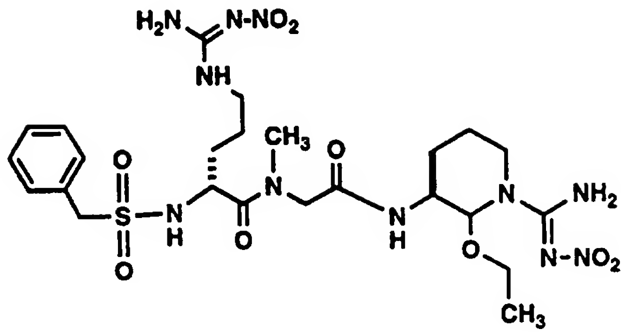
Preparation of benzylsulfonyl-(D)-N^g-NO_2-arginine sarcosine



To a solution of the compound of Example 12 (7.5 g, 14.8 mmole) in 30 mL of dry dimethylformamide and 45 mL acetonitrile was added alpha-toluenesulfonyl chloride (4.2 g, 22.3 mmole) followed by diisopropylethylamine (12.9 mL, 74.3 mmole). The mixture was stirred for 16 hours at room temperature, then piperidine (7.3 mL, 74.2 mmole) was added to remove the fluorenylmethyl group. After another 12 hours, the reaction mixture was dissolved in 600 mL of ethyl acetate and extracted into saturated aqueous sodium bicarbonate (2x200 mL). The combined aqueous fractions were washed with 200 mL ethyl acetate and then acidified to about pH 4 using concentrated hydrochloric acid. This was then extracted with ethyl acetate (2x300 mL) and the organic fractions were washed with 300 mL brine, dried over anhydrous magnesium sulfate, filtered and the solvent removed *in vacuo* to provide 3.28 g (50% yield) of the title compound as an off-white foam. Rf = 0.15 (1:9 methanol:dichloromethane).

Example 14

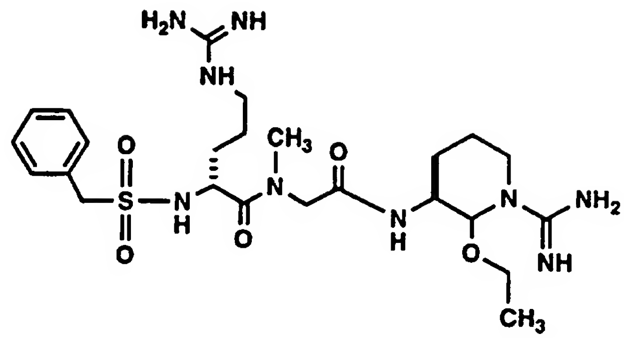
Preparation of benzylsulfonyl-(D)-N^g-NO_2-arginine-sarcosine-N^g-NO_2-arginine cyclic-OEt aminal



The compound of Example 4 (2.25 g, 8.42 mmole) and the compound of Example 13 (3.12 g, 7.02 mmole) were dissolved with stirring in 10 mL of dry dimethylformamide and 30 mL of dry acetonitrile. To this mixture was added 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride salt (2.02 g, 10.5 mmole) and 1-hydroxybenzotriazole monohydrate (1.42 g, 10.5 mmole), followed by diisopropylethylamine (6 mL, 4.5 mmole). After 16 hours, the reaction mixture was concentrated *in vacuo* then diluted with 600 mL ethyl acetate and extracted with 150 mL each of water, 1 M aqueous hydrochloric acid, water, saturated aqueous sodium bicarbonate and brine. The organic phase was dried over anhydrous magnesium sulfate, filtered and the solvent removed *in vacuo* to give 3.29 g (71% yield) of the title compound as an off-white foam. TLC gave two spots, Rf = 0.40 and 0.50 (1:9 methanol:dichloromethane).

Example 15

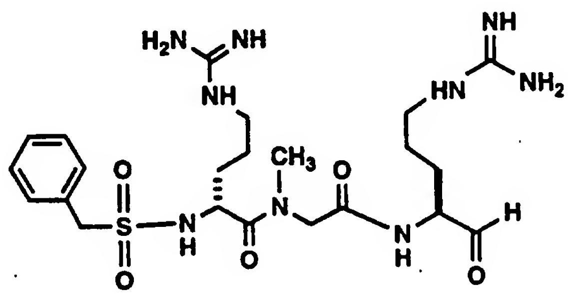
Preparation of benzylsulfonyl-(D)-arginine-sarcosine-arginine cyclic-OEt aminal



1 g of 10% palladium on carbon was placed in a 500 mL PARR bottle. To this was added 10 mL of water and 10 mL of glacial acetic acid. To this mixture was added a solution of the compound of Example 14 (3.29 g, 5 mmole) in 100 mL of ethanol. The mixture was then shaken under a hydrogen atmosphere at 40 psi for 5 days. (The catalyst was removed and replaced 2 times). The catalyst was then removed by filtration and the filtrate concentrated *in vacuo*. The resulting oil was azeotroped with toluene to remove the remaining acetic acid to afford about 2.8 g (quantitative yield) of the title compound.

Example 16

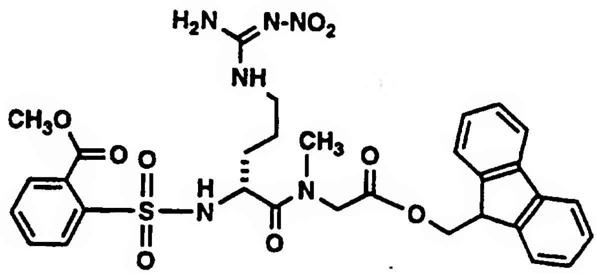
Preparation of benzylsulfonyl-(D)-arginine-sarcosine-arginine aldehyde



The compound of Example 15 (2.8 g, 5 mmole) was dissolved in 40 mL water and cooled to $0^{o}C$ in an ice water bath with stirring. To this solution was slowly added 40 mL of concentrated hydrochloric acid. After 1.5 hour, the reaction was judged complete by HPLC and the pH of the reaction mixture was adjusted to about pH 4 using saturated aqueous sodium acetate. This mixture was filtered through a plug of Celite. The title compound was obtained by purification from the filtrate by preparative HPLC (2 inch Vydak C18 column using a gradient consisting of 5 to 17% acetonitrile in water containing 0.1% trifluoroacetic acid run over 55 minutes at a flowrate of 115 mL/minute) and lyophilization of the pooled fractions. Mass Spectroscopy (FAB) confirmed the calculated molecular weight of 539.6.

Example 17

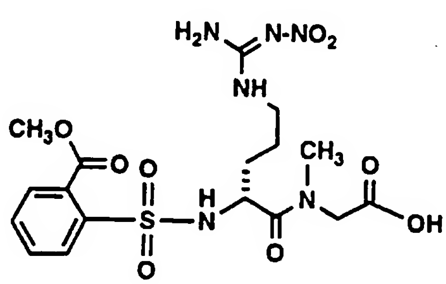
Preparation of (2-carbomethoxy)-benzenesulfonyl-(D)-N^g-nitroargininyl-sarcosine-O-fluorenylmethyl ester



Collidine (0.99 mL, 7.5 mmole) was added to suspension of the compound of Example 12 (1.5 g, 3.0 mmole) and 2-carbomethoxybenzenesulfonyl chloride (0.77 g, 3.3 mmole) in acetonitrile (25 mL) at room temperature. After stirring for 18 hours, distilled water (30 mL) was added, the reaction mixture was concentrated under vacuum, and the residue was extracted into ethyl acetate (2x50 mL), washed with 3% aqueous hydrochloric acid (50 mL), brine (50 mL), dried over anhydrous magnesium sulfate, and concentrated under vacuum to yield the title compound as a pale yellow foam (1.7 g, 84%). Rf=0.81 (9:1 dichloromethane:methanol).

Example 18

Preparation of (2-carbomethoxy)benzenesulfonyl-(D)-N^g-nitroargininyl-sarcosine

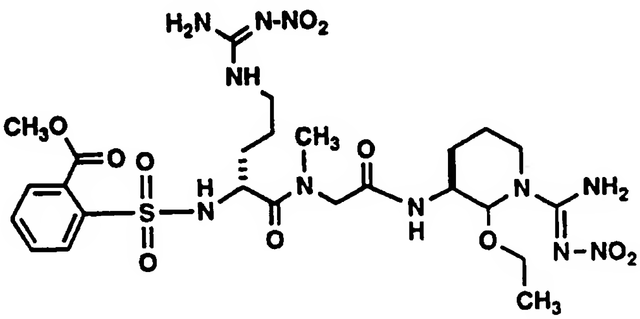


Piperidine (2.5 mL, 25 mmole) was added to a solution of the compound of Example 17 (1.7 g, 2.5 mmole) in

acetonitrile (20 mL). After stirring 1.5 hours at room temperature, the reaction was concentrated under vacuum and the residue was diluted with ethyl acetate (50 mL) extracted with aqueous sodium bicarbonate (2x50 mL), acidified with concentrated hydrochloric acid to pH 1, extracted into ethyl acetate, washed with brine, dried over anhydrous magnesium sulfate, and concentrated under vacuum to yield the title compound as a white foam (1.0 g, 80%). Rf=0.10 (9:1 dichloromethane:methanol).

Example 19

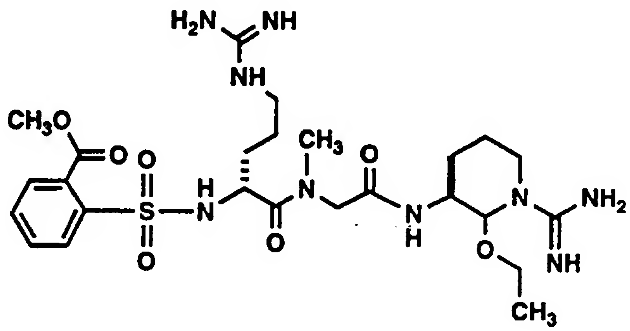
Preparation of (2-carbomethoxy)benzenesulfonyl-(D)-N^g-nitroargininyl-sarcosine-N^g-nitroarginine ethyl aminal



The compound of Example 18 (1.0 g, 2.0 mmole) and the compound of Example 4 (0.66 g, 2.5 mmole) were suspended in acetonitrile (20 mL), followed by 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride salt (0.48 g, 2.5 mmole) and 1-hydroxybenzotriazole monohydrate (0.34 g, 2.5 mmole). The reaction mixture was stirred for 30 minutes, then N-methylmorpholine (0.70 mL, 6.0 mmole) was added. After stirring for 48 hours at room temperature, the reaction mixture was concentrated under vacuum, and the residue was extracted into ethyl acetate (2x50 mL), washed with 3% aqueous hydrochloric acid (50 mL), brine (50 mL), dried over anhydrous magnesium sulfate, and concentrated under vacuum to yield the title compound as a pale yellow foam (0.77 g, 55%). Rf=0.73 (9:1 dichloromethane:methanol).

Example 20

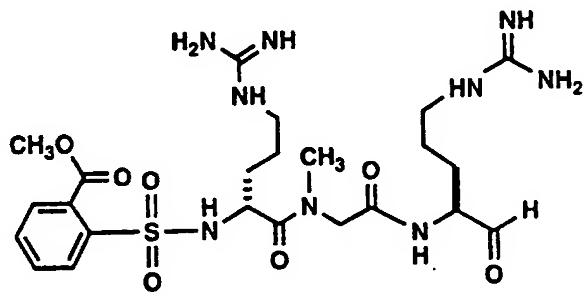
Preparation of (2-carbomethoxy)benzenesulfonyl-(D)-argininyl-sarcosine-arginine ethyl aminal



The compound of Example 19 (0.77 g, 1.1 mmole) was dissolved in methanol (20 mL), acetic acid (5.0 mL), and distilled water (5.0 mL), and the solution was placed in a 250 mL Parr bottle. The vessel was purged with argon and then 10% palladium on carbon catalyst (0.3 g) was added. The reaction mixture was shaken under hydrogen (50 psi) for 2.5 days, then was filtered through Celite and concentrated under vacuum to give the title compound as an off-white foam.

Example 21

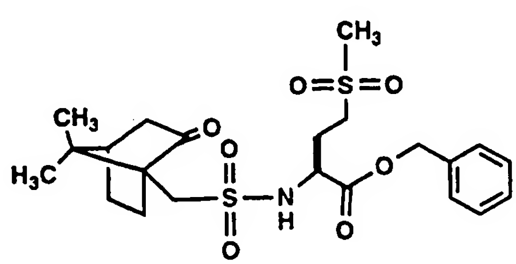
Preparation of (2-carbomethoxy)benzenesulfonyl-(D)-argininyl-sarcosine-argininal



The compound of Example 20 (0.3 g, 0.49 mmole) was dissolved in 6N hydrochloric acid (8.0 mL, 48 mmole) at 0°C. The reaction mixture was stirred at 0°C for 4 hours, then at room temperature for 1.5 hours. The reaction mixture was recooled to 0°C, and saturated sodium acetate (20 mL) was added (to pH 4). Purification on reverse phase HPLC, followed by lyophilization, gave the product as a white powder. Fast atom bombardment mass spectrometry confirmed the theoretical molecular weight of 583.

Example 22

Preparation of (D)-camphorsulfonyl-L-methionine sulfone-O-benzyl ester

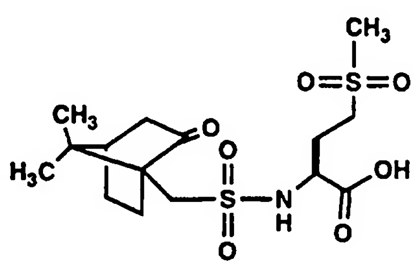


To a solution of L-methionine sulfone-O-benzyl ester hydrochloride salt (3.86 g, 12.5 mmole) in 120 mL of dry 50:50 dimethylformamide:tetrahydrofuran, was added D-camphorsulfonyl chloride (4.7 g, 18.7 mmole) followed by

triethylamine (8.7 mL, 62.5 mmole). The mixture was stirred for 16 hours at room temperature. The reaction mixture was dissolved in 800 mL of ethyl acetate and washed with 300 mL each of water, 1 M aqueous hydrochloric acid, water, saturated aqueous sodium bicarbonate and brine. The organic phase was dried over anhydrous magnesium sulfate, filtered and the solvent removed *in vacuo*. The resulting oil was dissolved in dichloromethane and filtered through silica, eluting first with dichloromethane (500 mL) then 1:9 methanol:dichloromethane (1000 mL). The methanol:dichloromethane fraction was concentrated to provide 5.4 g (88% yield) of the title compound as an off-white foam. Rf = 0.65 (1:9 methanol:dichloromethane).

Example 23

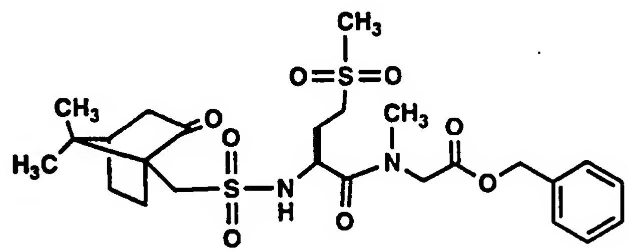
Preparation of (D)-camphorsulfonyl-L-methionine sulfone



To a solution of the compound of Example 22 (5.4 g, 11.1 mmole) in 400 mL of methanol under a nitrogen blanket, was added 10% palladium on carbon (2.5 g). The mixture was hydrogenated at 1 atmosphere for 16 hours. The mixture was then filtered and concentrated *in vacuo* to provide 4.1 g (94% yield) of the title compound as a white foam. Rf = 0.2 (1:9 methanol:dichloromethane).

Example 24

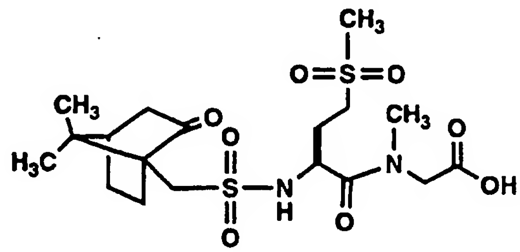
Preparation of (D)-camphorsulfonyl-L-methionine sulfone-sarcosine-O-benzyl ester



To a solution of the compound of Example 23 (3.7 g, 9.4 mmole) in 47 mL of dimethylformamide, was added with stirring sarcosine-O-benzyl ester para-toluenesulfonate salt (3.3 g, 9.4 mmole), followed by N-methylmorpholine (5.1 mL, 47 mmole) and benzotriazol-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (5.4 g, 9.4 mmole). The stirring was continued for 16 hours. The reaction mixture was dissolved in 700 mL of ethyl acetate and washed with 250 mL each of water, 1 M aqueous hydrochloric acid, water, saturated aqueous sodium bicarbonate and brine. The organic phase was dried over anhydrous magnesium sulfate, filtered and the solvent removed in vacuo to provide 5.16 g (96% yield) of the title compound as an off-white foam. Rf = 0.3 (1:9 methanol:dichloromethane).

Example 25

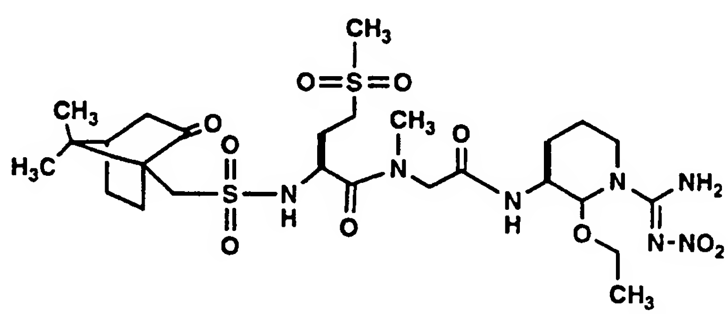
Preparation of (D)-camphorsulfonyl-L-methionine-sulfone sarcosine



To a solution of the compound of Example 24 (5.16 g, 9.01 mmole) in 300 mL of methanol under a nitrogen blanket, was added 10% palladium on carbon (4 g) and the mixture was hydrogenated at 1 atmosphere for 16 hours. The mixture was then filtered and concentrated *in vacuo* to provide 3.79 g (88% yield) of the title compound as a white foam. Rf = 0.2 (1:9 methanol:dichloromethane).

Example 26

Preparation of (D)-camphorsulfonyl-L-methionine sulfone-sarcosine-N^g-NO_2-arginine cyclic-OEt aminal

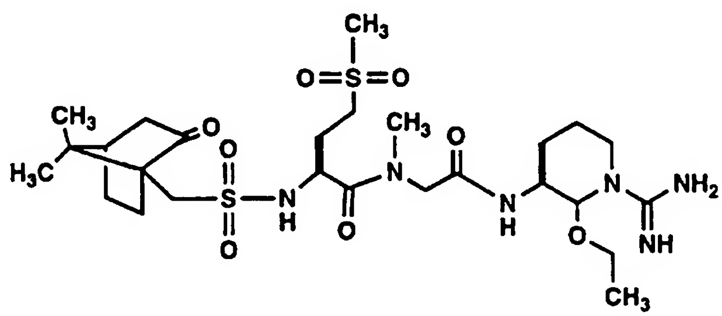


The compound of Example 4 (0.85 g, 3.1 mmole) was dissolved with stirring in 6 mL of dry dimethylformamide and 16 mL of dry acetonitrile. To this mixture was added N-methylmorpholine (1.7 mL, 15.8 mmole) followed by the compound of Example 25 (1.15 g, 2.38 mmole), 1-hydroxybenzotriazole monohydrate (0.65 g, 4.7 mmole) and

2-(1H-benzotriazol-1-yl)-1,1,3,3-t tramethyluronium hexafluorophosphate (1.8 g, 4.7 mmole). After 16 hours, the reaction mixture was diluted with 600 mL ethyl acetate and extracted with 200 mL each of water, 1 M aqueous hydrochloric acid, water, saturated aqueous sodium bicarbonate and brine. The organic phase was dried over anhydrous magnesium sulfate, filtered and the solvent removed *in vacuo*. Chromatography of the resulting oil on silica eluting with 4:1:4 hexanes:methanol:dichloromethane afforded 0.61 g (35% yield) of the title compound as an off-white foam. Rf = 0.45 (1:9 methanol:dichloromethane).

Example 27

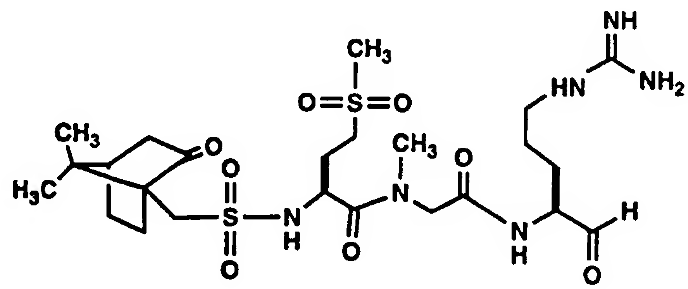
Preparation of (D)-camphorsulfonyl-L-methionine sulfone-sarcosine-arginine cyclic-OEt aminal



0.5 g of 10% palladium on carbon was placed in a 500 mL PARR bottle. To this was added 6 mL of water and 2 mL of glacial acetic acid. To this mixture was added a solution of the compound of Example 26 (0.61 g, 0.84 mmole) in 60 mL of methanol. The mixture was then shaken under a hydrogen atmosphere at 40 psi for 3 days. The catalyst was then removed by filtration and the filtrate concentrated *in vacuo*. The resulting oil was azeotroped with toluene to remove the remaining acetic acid to afford 0.5 g (87% yield) of the title compound.

Example 28

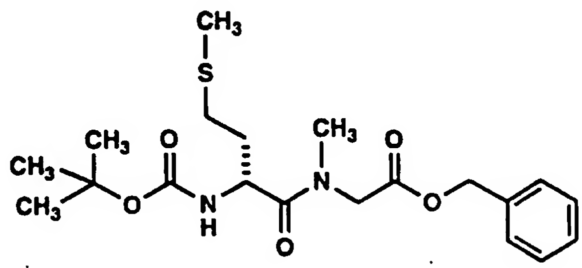
<u>Preparation of (D)-camphorsulfonyl-L-methionine sulfone-sarcosine-arginine aldehyde</u>



The compound of Example 27 (0.5 g, 0.73 mmole) was dissolved in 20 mL of 50:50 water:acetonitrile with stirring and cooled to 0°C in an ice water bath. To this solution was slowly added 30 mL of a 60 wt% solution of hexafluorophosphoric acid in water. After 1 hour, the pH of the reaction mixture was adjusted to about pH 4 using saturated aqueous sodium acetate. This mixture was filtered through a plug of Celite. The title compound was obtained by purification from the filtrate by preparative HPLC (2 inch Vydak C18 column using a gradient consisting of 15-35% acetonitrile in water containing 0.1% trifluoroacetic acid run over 40 minutes at a flowrate of 115 mL/minute) and lyophilization of the pooled fractions. Mass Spec(FAB) confirmed the calculated molecular weight of 605.7.

Example 29

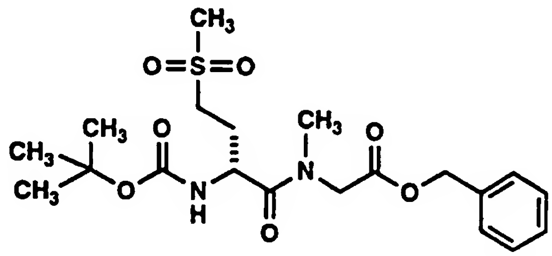
Preparation of N-Boc-(D)-methioninyl sarcosine benzyl ester



N-Boc-(D)-methionine (17.1 g, 68.58 mmole) and sarcosine benzyl ester tosylate salt (24.08 g, 68.60 mmole) were suspended in 110 mL of acetonitrile and 25 mL of dimethylformamide at 0°C, then benzotriazol-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (30.34 g, 68.58 mmole) and N-methylmorpholine (20.83 g, 205.83 mmole) were added. The ice bath was removed after 30 minutes and the reaction was stirred for 18 hours at room temperature. The reaction mixture was reduced in volume under vacuum at 25°C to give an oil. The oil was dissolved in ethyl acetate (250 mL), then successively washed with 1N hydrochloric acid (1x50 mL), saturated sodium bicarbonate (1x50 mL) and brine (1x50 mL). The organic layer was dried with anhydrous magnesium sulfate and evaporated under vacuum to give crude product. The crude product was purified by column chromatography on silica gel, eluting with 60:40 hexane:ethyl acetate to yield 26.07 g (92.6 %) of the title compound as an oil. Thin layer chromatography analysis of the title compound showed a single spot with Rf = 0.55 (silica, 3:2 ethyl acetate/hexane).

Example 30

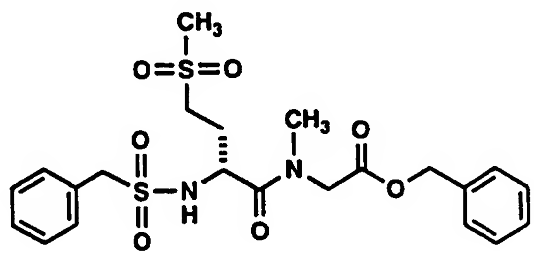
Preparation of N-Boc-(D)-methioninylsulfone sarcosine benzyl ester



The compound of Example 29 (26.0 g, 63.3 mmole) was dissolved in 335 mL of glacial acetic acid. Sodium perborate tetrahydrate (48.7 g, 316.5 mmole) was added and the mixture was heated to 55°C. After 2.5 hours at this temperature, the reaction mixture was diluted with 1.1 liters of brine, the aqueous layer was extracted with ethyl acetate (4x250 mL) and the combined organic extracts were dried with anhydrous magnesium sulfate. This solution was filtered and evaporated under vacuum, then repeatedly azeotroped with toluene (200 mL) under vacuum to remove acetic acid. The residual slurry was dissolved in ethyl acetate (200 mL), filtered and the filtrate evaporated to yield 24.15 g (86.2 %) of the title compound as a white solid. Thin layer chromatography analysis of the title compound showed a single spot with Rf = 0.30 (silica, 2:3 ethyl acetate: hexane).

Example 31

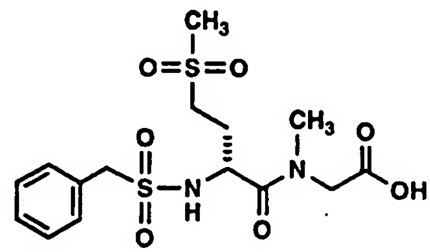
Preparation of N-benzylsulfonyl-(D)-methioninylsulfone sarcosine benzyl ester



A solution of the compound of Example 30 (7.0 g, 15.8 mmole) in 5 mL of dichloromethane was prepared. 27 mL of 4M hydrochloric acid in dioxane was added and the mixture was stirred for several hours at room temperature until all starting material was consumed. The mixture was evaporated under vacuum and the resulting oil was dissolved in acetonitrile and then evaporated under vacuum. This was done three times. The remaining oil was suspended in 17 mL of acetonitrile and 5 mL of dimethylformamide, cooled to ice bath temperature, then benzylsulfonyl chloride (5.28 g, 23.7 mmole) and N-methylmorpholine (4.80 g, 47.5 mmole) were added. The reaction mixture was removed from the ice bath after 30 minutes and stirred at room temperature for 18 hours. The reaction mixture was reduced in volume under vacuum to an oil. The oil was taken up in 200 mL ethyl acetate and washed successively with 1N hydrochloric acid (1x50 mL), saturated sodium bicarbonate (1x50 mL) and brine (1x50 mL). After drying with anhydrous magnesium sulfate, the organic layer was evaporated under vacuum to give crude product. The crude product was purified by column chromatography on silica gel, eluting with 3:7 hexane:ethyl acetate to yield 4.42 g (56.3 % yield) of the title compound as a solid. Thin layer chromatography analysis of the title compound showed a single spot with Rf = 0.70 (silica, 95:5 dichloromethane:methanol).

Example 32

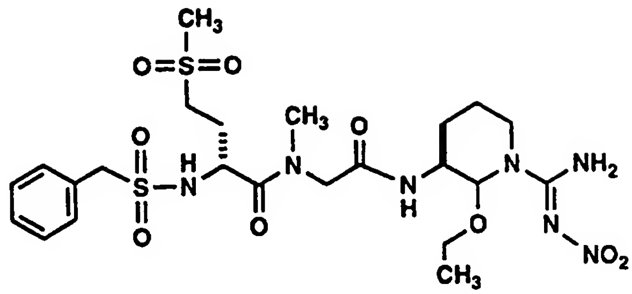
Preparation of N-benzylsulfonyl-(D)-methioninylsulfone sarcosine acid



The compound of Example 31 (4.42 g, 8.9 mmole) was dissolved in tetrahydrofuran (200 mL), 0.8 g of 10% palladium on carbon was added and the mixture was stirred under hydrogen gas at atmospheric pressure for 18 hours. After the catalyst was filtered off the reaction mixture, the solvent was removed under vacuum and the resulting oil was taken up in a solution of saturated sodium bicarbonate. This solution was then extracted with ethyl acetate (1x150 mL) and the organic layer was decanted off. The remaining aqueous layer was layered with 100 mL of ethyl acetate and acidified with 3N hydrochloric acid to pH 2 (pH papers). After the phases separated, the organic layer was saved and the aqueous layer was then further extracted with ethyl acetate (3x100 mL). The organic extracts were combined and washed with brine, dried with anhydrous magnesium sulfate, filtered and evaporated under vacuum to give 3.17 g (yield 87.6 %) of the title compound as a foamy solid. Thin layer chromatography analysis of the title compound showed a single spot with Rf = 0.50 (silica, 90:10:2 dichloromethane:methanol:acetic acid).

Example 33

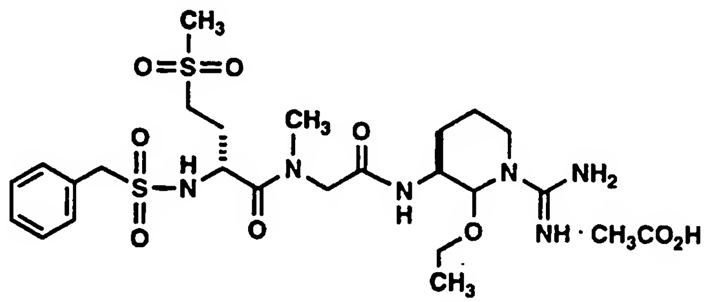
Preparation of N-benzylsulfonyl-(D)-methioninylsulfone sarcosine nitroarginine ethyl aminal



The compound of Example 32 (1.22 g, 3 mmole) and the compound of Example 4 (1.6 g, 6 mmole) were suspended in 15 mL of acetonitrile and 9 mL of dimethylformamide, then 1-hydroxybenzotriazole monohydrate (0.61 g, 4.5 mmole), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (1.71 g, 4.5 mmole) and N-methylmorpholine (1.5 g, 15 mmole) were added. The reaction mixture was stirred for 3 days at room temperature. The reaction mixture was reduced in volume under vacuum at 25°C to give an oil. The oil was dissolved in ethyl acetate (600 mL), then successively washed with 1N hydrochloric acid (1x150 mL), saturated sodium bicarbonate (1x150 mL) and brine (1x150 mL). The organic layer was dried with anhydrous magnesium sulfate and evaporated under vacuum to give crude product. The crude product was purified by column chromatography on silica gel, eluting with 97.5:2.5 dichloromethane:methanol to yield 0.6 g (32.3 %) of the title compound as a solid. Thin layer chromatography analysis of the title compound showed a single spot with Rf 0.60 (silica, 90:10:2 dichloromethane:methanol:acetic acid).

Example 34

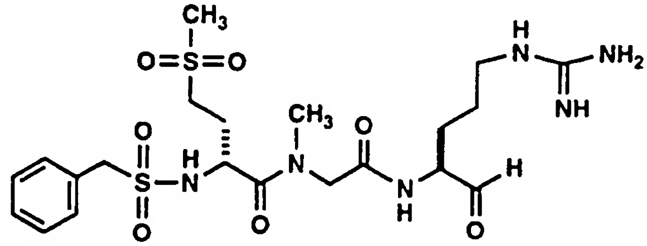
Preparation of N-benzylsulfonyl-(D)-methioninylsulfone sarcosine arginine ethyl aminal, acetate salt



The compound of Example 33 (0.50 g, 0.80 mmole) was dissolved in 40 mL of methanol, 7 mL of water and 0.3 mL of glacial acetic acid. 0.25 g of 10% Palladium on carbon catalyst was added to the solution and the mixture was placed on a PARR shaker at 40 psi hydrogen for 18 hours at room temperature, at which point the starting material was consumed. The catalyst was filtered and the reaction solution was reduced in volume under vacuum at 25°C to leave an oil. Toluene was added and evaporated several times, then methanol was added and evaporated to yield 0.48 g (95 %) of the title compound as a white solid.

Example 35

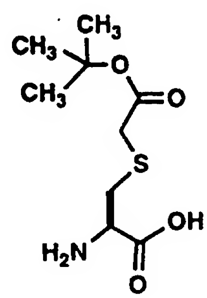
Preparation of N-benzylsulfonyl-methioninylsulfone sarcosine argininal



The compound of Example 34 (0.48 g, 0.75 mmole) was placed in a plastic reaction vessel and dissolved by addition of 5 mL of acetonitrile and 5 mL of deionized water. The solution was cooled to 0°C, then 10 mL of hexafluorophosphoric acid (60% wt. in water) at 0°C was added. The reaction mixture was stirred at 0°C for 2.0 hours at which time all the starting material was consumed. The reaction was quenched with 100 mL of 2.5 M sodium acetate, this raised the pH to pH 5. The title compound was isolated by preparative HPLC purification (2 inch Vydak C-18 at 115 mL/minute, gradient 6 to 25% acetonitrile in water containing 0.1% TFA run over 50 minutes). Mass Spectroscopy (FAB) confirmed the calculated molecular weight of 546.6.

Example 36

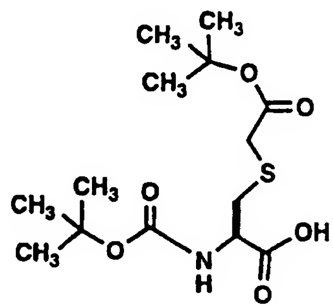
Preparation of S-(t-butyl acetate)-L-cysteine



A 360 mL aqueous solution of L-cysteine hydrochloride monohydrate (60.0 g, 341.7 mmole) and sodium hydroxide (27.33 g, 683.4 mmole) at room temperature was treated with a solution of t-butyl bromoacetate (72.3 g, 370.6 mmole) in 130 mL of dioxane over 30 minutes. This reaction mixture was stirred for 18 hours, during which time a thick precipitate formed. The solid was filtered off, washed with diethyl ether (100 mL) and dried under high vacuum at 40°C to give 82.5 g (103.8% crude yield includes occluded inorganic salt) of the title compound.

Example 37

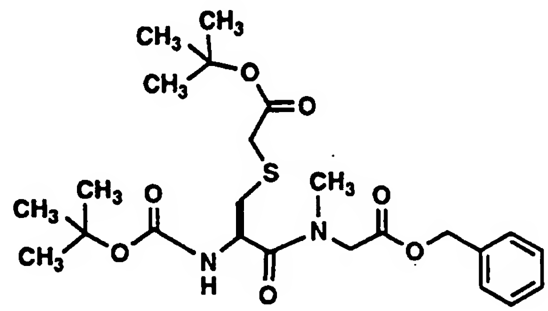
Preparation of N-Boc-S-(t-butyl acetate)-L-cysteine



The compound of Example 36 (82.5 g, 341.7 mmole) and sodium bicarbonate (33.96 g, 404 mmole) were suspended in 600 mL of deionized water. A solution of di-t-butyl dicarbonate (80.88 g, 370 mmole) in 350 mL of dioxane was added and the slurry was stirred for 18 hours. The slurry was extracted with diethyl ether (2x100 mL). The slurry was layered with ethyl acetate (200 mL) and acidified with 1N hydrochloric acid to pH 2 (pH papers). The resulting organic layer was saved and the remaining aqueous layer was further extracted with ethyl acetate (2x200 mL). The organic extracts were combined, washed with brine, dried with anhydrous magnesium sulfate and the solvent evaporated under vacuum to yield 84.3 g (74.6 %) of the title compound as a clear oil. Thin layer chromatography analysis of the title compound showed a single spot with Rf = 0.55, (silica; 90:10:2 dichloromethane:methanol: acetic acid).

Example 38

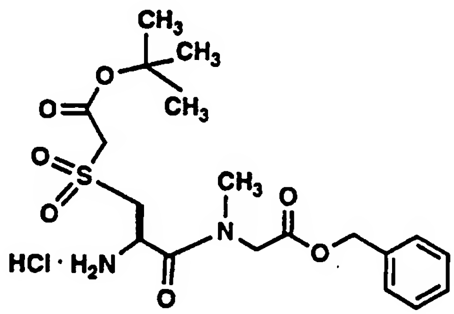
Preparation of N-Boc-S-(t-butyl acetate)-L-cysteine sarcosine-O-benzyl ester



The compound of Example 37 (57.32 g, 170.9 mmole) and sarcosine benzyl ester tosylate salt (60.0 g, 170.9 mmole) were suspended in 300 mL of acetonitrile and 60 mL of dimethylformamide at 0°C, then benzotriazol-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (75.2 g, 170.9 mmole) and N-methylmorpholine (51.9 g, 512.7 mmole) were added. The ice bath was removed after 30 minutes and the reaction was stirred for 48 hours at room temperature. The reaction mixture was reduced in volume under vacuum at 25°C to give an oil. The oil was dissolved in ethyl acetate (250 mL), then successively washed with 1N hydrochloric acid (1x50 mL), saturated sodium bicarbonate (1x50 mL) and brine (1x50 mL). The organic layer was dried with anhydrous magnesium sulfate and evaporated under vacuum to give crude product. The crude product was purified by column chromatography on silica gel, eluting with 60:40 hexane:ethyl acetate to yield 68.1 g (80.2 %) of the title compound as an oil. Thin layer chromatography analysis of the title compound showed a single spot with Rf = 0.64 (silica, 3:2 ethyl acetate:hexane).

Example 39

Preparation of S-(t-butyl acetate)-L-cysteine sulfone sarcosine-O-benzyl ester, hydrochloride salt



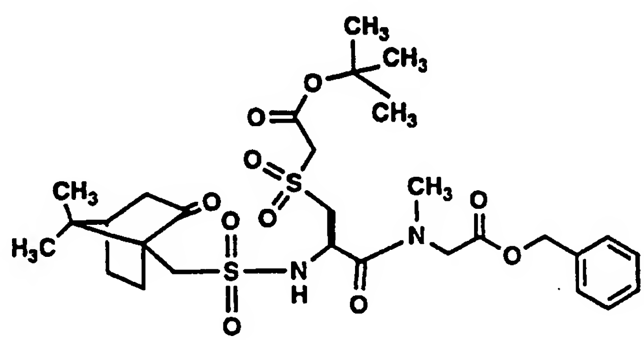
The compound of Example 38 (55.6 g, 111.96 mmole) was dissolved in 580 mL of glacial acetic acid and then sodium perborate tetrahydrate (86.1 g, 559.8 mmole) was added. This mixture was heated to 55°C. After 2.5 hours at this temperature, the reaction mixture was diluted with 1 liter of brine, the aqueous layer was extracted with ethyl acetate (4x250 mL), and the combined organic extracts were dried with anhydrous magnesium sulfate. This solution was filtered and evaporated under vacuum, then repeatedly azeotroped with toluene (200 mL) under vacuum to remove acetic acid. The residual slurry was dissolved in ethyl acetate (200 mL), filtered and the filtrate evaporated to yield 50.6 g (85.5 %) of N-Boc-S-(t-butyl acetate)-L-cysteine sulfone sarcosine-O-benzyl ester as a white solid. Thin layer chromatography analysis of the title compound showed a single spot with Rf = 0.50 (silica, 3:2 ethyl acetate:hexane).

To a cooled (0°C) solution of acetyl chloride (48.6 mL, 683 mmole) in dry ethyl acetate (133 mL) was slowly added with stirring dry methanol (27.7 mL, 683 mmole). After 10 minutes, the reaction mixture was allowed to warm to room temperature for 30 minute and then recooled to 0°C. To the reaction mixture was slowly added a solution of N-Boc-S-(t-butyl acetate)-L-cysteine sulfone sarcosine-O-benzyl ester (20.78 g, 42.7 mmole) in dry ethyl acetate

(1000 mL). After 4.5 hours at reduced temperature, the reaction was deemed complete by TLC (1:9 methanol:methylene chloride). The reaction was concentrated *in vacuo* to about 100 mL and the title compound precipitated by pouring into stirring diethyl ether (600 mL). The titled compound was isolated by filtration and dried in vacuo to afford 11.5 g (61% yield) of a white powder.

Example 40

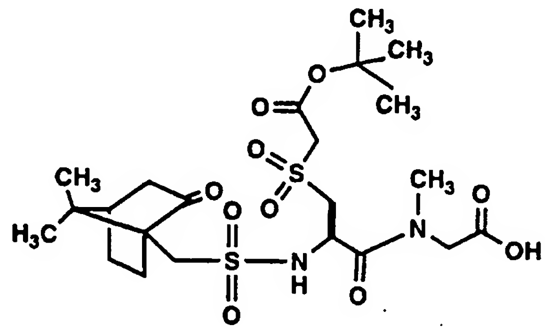
Preparation of N-(D)-camphorsulfonyl-S-(t-butyl acetate)-L-cysteine sulfone sarcosine-O-benzyl ester



The compound of Example 39 (3.9 g, 9.22 mmole) and (D)-camphorsulfonyl chloride (3.5 g, 13.8 mmole) were dissolved with stirring in 20 mL of dry dimethylformamide and 20 mL of dry tetrahydrofuran. To this mixture was added triethylamine (6.4 mL, 46.1 mmole). After 16 hours, the reaction mixture was diluted with 600 mL of ethyl acetate and washed with 150 mL each of water, 1 M aqueous hydrochloric acid, water, saturated aqueous sodium bicarbonate and brine. The organic phase was dried over anhydrous magnesium sulfate, filtered and concentrated in vacuo to afford 3.89 g (70% yield) of the title compound as a yellowish foam. Rf = 0.84 (1:9 methanol:dichloromethane).

Example 41

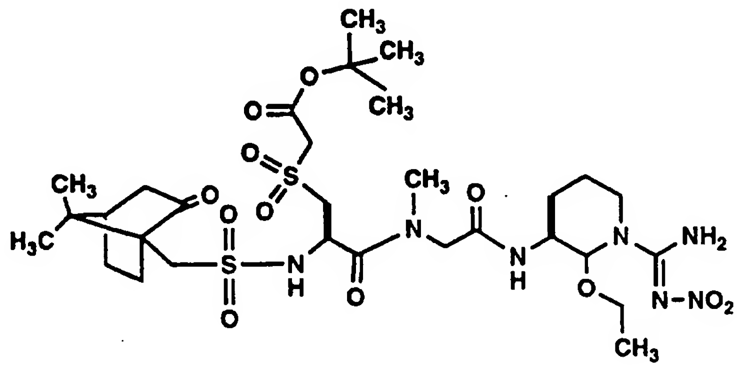
Preparation of N-(D)-camphorsulfonyl-S-(t-butyl acetate)-L-cysteine sulfone sarcosine



The compound of Example 40 (3.89 g, 6.49 mmole) was dissolved in 300 mL of methanol with stirring and purged with nitrogen. To this mixture was added 2 g of 10% palladium on carbon and stirred vigorously under 1 atmosphere of hydrogen. After 16 hours, the palladium was removed by filtration and the solvent removed *in vacuo*. The resulting oil was dissolved into 100 mL of ethyl acetate and the product was extracted into 150 mL saturated aqueous sodium bicarbonate. This was washed with 100 mL ethyl acetate and acidified using concentrated hydrochloric acid. The product was then extracted into ethyl acetate (3x200 mL) and the combined organic layers were washed with 200 mL brine and dried over anhydrous magnesium sulfate, filtered and concentrated *in vacuo* to 1.9 g (57% yield) of the title compound as a white foam.

Example 42

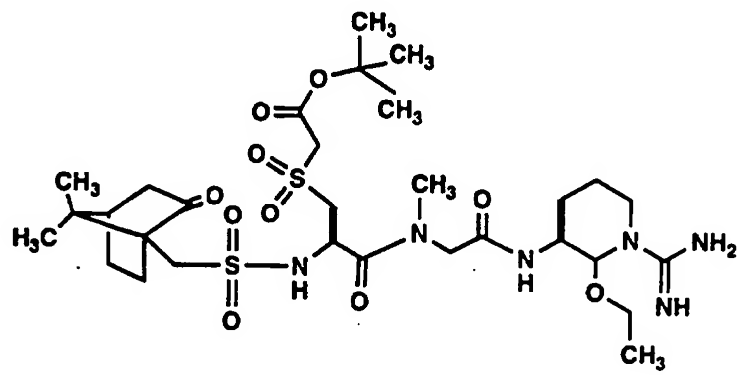
Preparation of (D)-camphorsulfonyl-s-(t-butylacetate) cysteinesulfone sarcosine-N^g-NO_2-arginine cyclic-OEt aminal



The compound of Example 4 (1.50 g, 5.59 mmole) and the compound of Example 41 (1.9 g, 3.72 mmole) were dissolved with stirring in 10 mL of dry dimethylformamide and 20 mL of dry tetrahydrofuran. To this mixture was added 1-hydroxybenzotriazole monohydrate (0.76 g, 5.6 mmole) and 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (2.12 g, 5.6 mmole) followed by N-methylmorpholine (2 mL, 19 mmole). After 16 hours, the reaction mixture was diluted with 600 mL ethyl acetate and extracted with 150 mL each of water, 1 M aqueous hydrochloric acid, water, saturated aqueous sodium bicarbonate and brine. The organic phase was dried over anhydrous magnesium sulfate, filtered and the solvent removed *in vacuo*. Chromatography of the resulting oil (silica, 4:1:4 hexanes:methanol: dichloromethane) afforded 1.16 g (43% yield) of the title compound as an off-white foam. TLC gave two spots, Rf = 0.40 and 0.47 (1:9 methanol:dichloromethane).

Example 43

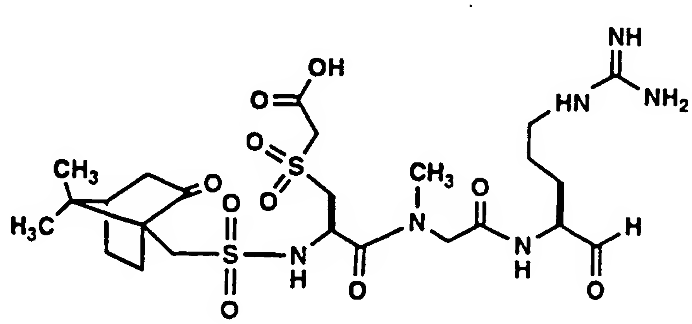
Preparation of (D)-camphorsulfonyl cysteinesulfone-s-(t-butylacetate) sarcosine arginine cyclic-OEt aminal



1 g of 10% palladium on carbon was placed in a 500 mL PARR bottle. 10 mL of water and 3 mL of glacial acetic acid was added. To this mixture was added a solution of the compound of Example 42 (1.16 g, 1.6 mmole) in 100 mL of methanol. The mixture was then shaken under a hydrogen atmosphere at 40 psi for 1 day. The catalyst was then removed by filtration and the filtrate concentrated *in vacuo*. The resulting oil was azeotroped with toluene to remove the remaining acetic acid to afford about 1 g of the title compound.

Example 44

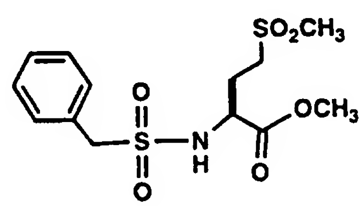
Preparation of (D)-camphorsulfonyl-s-(acetic acid) cysteinesulfone sarcosine arginine aldehyde



The compound of Example 43 (1 g, 1.6 mmole) was dissolved in 30 mL of 50:50 water:acetonitrile with stirring and cooled to 0°C in an ice water bath. To this solution was slowly added 50 mL of a 60 wt% solution of hexafluorophosphoric acid in water. After 1 hour, the pH of the reaction mixture was adjusted to about pH 4 using saturated aqueous sodium acetate. This mixture was filtered through a plug of Celite. The title compound was obtained by purification from the filtrate by preparative HPLC (2 inch Vydak C18 column using a gradient consisting of 8 to 27% acetonitrile in water containing 0.1% trifluoroacetic acid run over 60 minutes at a flowrate of 115 mL/minute) and lyophilization of the pooled fractions. Mass Spectroscopy (FAB) confirmed the calculated molecular weight of 635.7.

Example 45

Preparation of benzylsulfonylmethionine(sulfone) methyl ester

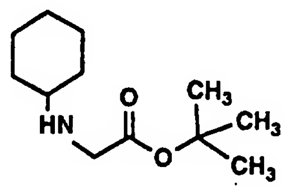


Carbonyldiimidazole (4.9 g, 30 mmole) was added to a suspension of benzylsulfonylmethionine sulfone (8.0 g, 28.5 mmole) in dichloromethane (60 mL) at 0°C. The reaction mixture was allowed to warm to room temperature and was stirred an additional 17 hours. Methanol (2.3 mL, 57 mmole) was added and stirring was continued for 17 hours, at which time the reaction mixture was concentrated under vacuum. The residue was extracted into ethyl acetate (2x100 mL), washed with 3% hydrochloric acid (aq) (75 mL), brine (75 mL), saturated sodium bicarbonate solution (aqueous) (75 mL), dried over anhydrous magnesium sulfate, and concentrated under vacuum to yield the title

compound as a white foam (6.0 g, 71%). Rf=0.11; 1:1 ethyl acetate:hexanes.

Example 46

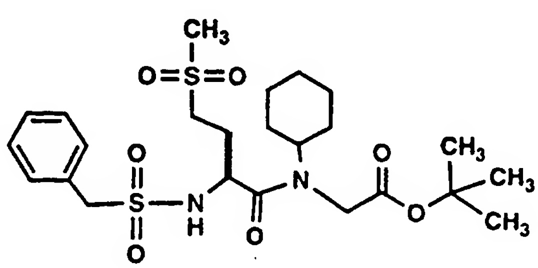
Preparation of t-butyl N-cyclohexylglycine



t-Butyl bromoacetate (7.6 mL) was added over 10 minutes to a solution of cyclohexylamine (11.7 mL) in tetrahydrofuran (200 mL) at 0°C. After the addition was complete, the ice-water bath was removed and the reaction was stirred at room temperature for 20 hours. The reaction was filtered through Celite and concentrated under vacuum. The product was purified by flash chromatography on silica gel eluting with a hexane-ethyl acetate (4:1 to 2:1) gradient yielding a clear oil (10.8 g, 99%). Rf=0.22 (1:1 ethyl acetate:hexanes).

Example 47

Preparation of benzylsulfonylmethioninyl(sulfone) N-cyclohexylglycine t-butyl ester

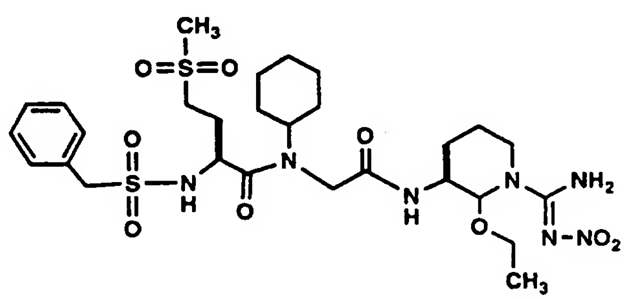


To a solution of the compound of Example 46 (5.0 g, 23.5 mmole) in tetrahydrofuran (40 mL), was added over 15 minutes a 2M solution of trimethylaluminum in toluene (11.5 mL, 23 mmole) at 0°C. After stirring for 1.3 hours,

benzylsulfonylmethionine sulfone methyl ester (1.4 g, 4.7 mmole) was added in tetrahydrofuran (10 mL) over 10 minutes. After the reaction mixture warmed to room temperature, it was stirred for 3 days. The reaction mixture was then poured into cold 3% hydrochloric acid (50 mL) and ethyl acetate (50 mL), separated, extracted with ethyl acetate (50 mL), washed with 3% hydrochloric acid (aq) (50 mL), brine (50 mL), dried over anhydrous magnesium sulfate, and concentrated under vacuum. Purification by silica gel chromatography using a dichloromethane, methanol gradient (2-5%) gave the title compound as an orange oil (0.36 g, 16%). Rf=0.91 (9:1 dichloromethane:methanol).

Example 48

Preparation of benzylsulfonylmethioninyl(sulfone)N-cyclohexylglycinyl-N^g-nitrocycloarginine ethyl aminal



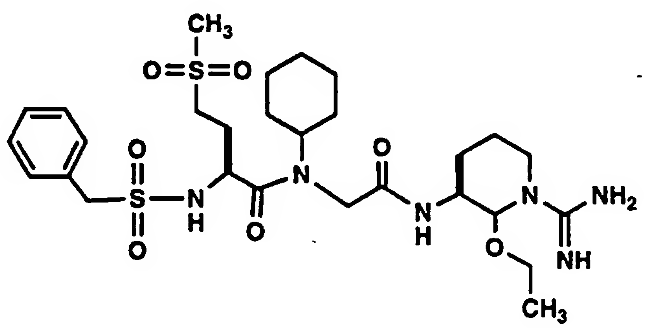
The compound of Example 47 (0.36 g, 0.76 mmole) was stirred with trifluoroacetic acid (5.0 mL) and dichloromethane (5.0 mL) at room temperature for 3.5 hours. Toluene (5.0 mL) was added and the reaction was concentrated under vacuum to give a residue.

The residue was dissolved in acetonitrile (5.0 mL), and the compound of Example 4 (0.223 g, 0.76 mmole), 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride salt (0.18 g, 0.91 mmole), 1-hydroxybenzotriazole monohydrate (0.12 g, 0.91 mmole), and finally N-methylmorpholine (0.25 g, 2.3 mmole) were added. The reaction mixture was stirred at room temperature for 20

hours, then was concentrated under vacuum to give a residue. The residue was then extracted into ethyl acetate (2x50 mL), washed with 3% hydrochloric acid (aq) (50 mL), brine (50 mL) sodium bicarbonate solution (aq) (50 mL), dried over anhydrous magnesium sulfate, and concentrated under vacuum to yield the product as a pale yellow foam (0.30 g, 62%). Rf=0.90 (9:1 dichloromethane:methanol).

Example 49

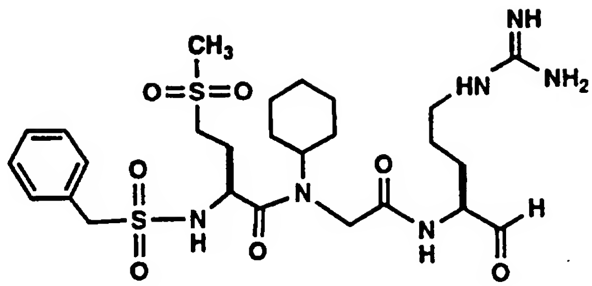
Preparation of benzylsulfonylmethioninyl(sulfone)N-cyclohexylglycinylcycloarginine ethyl aminal



The compound of Example 48 (0.30 g, 0.47 mmole) was dissolved in methanol (12 mL), acetic acid (2.0 mL), and distilled water (2.0 mL) and the solution was placed in a 250 mL Parr bottle. The reaction vessel was purged with argon and then 10% palladium on carbon catalyst (0.15 g) was added. The reaction mixture was shaken under hydrogen (45 psi) for 2.8 days, then was filtered through Celite and concentrated under vacuum to give crude title compound. This was purified by reverse phase HPLC on a two inch Vydak C-18 at 115 mL/minute, gradient 20-50% over 50 minutes and lyophilyzed to give the title compound as a white powder (0.2 g, 74%).

Example 50

Preparation of benzylsulfonylmethioninyl(sulfone)N-cyclohexylglycinylargininal

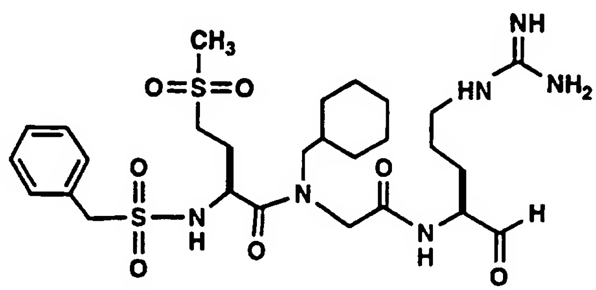


The compound of Example 49 (0.20 g, 0.34 mmole) was dissolved in acetonitrile (3.0 mL), cooled to 0°C using an ice bath, and 6N hydrochloric acid (8.0 mL) was added. The ice-water bath was removed and the reaction was stirred at room temperature for 3 hours. An additional (1.0 mL) concentrated hydrochloric acid was added. After 1.0 hour, saturated sodium acetate (15 mL) was added. The solution was then filtered and then subjected to purification by HPLC. The product was purified by reverse phase HPLC (2 inch Vydak C-18 at 115 mL/minute, gradient 13 to 45% acetonitrile in water containing 0.1% trifluoroacetic acid run over 40 minutes) and lyophilyzed to give the title compound as a white powder. Fast atom bombardment mass spectrometry confirmed the theoretical molecular weight of 614 based on formula of $C_{26}H_{42}N_6O_7S_2$.

Example 51

Preparation of other benzylsulfonylmethionyl(sulfone) N-alkylglycinylargininals

The procedures described in Examples 45 through 50, are used to prepare other preferred compounds of the present invention which are shown below. To prepare these compounds, other substituted amines are used in the place of cyclohexylamine in Example 46. For example, other substituted amines would include cyclohexylmethylamine, 4-hydroxycyclohexylamine, aniline, benzylamine, cyclopentylamine, isopropylamine, isobutylamine, R and S sec-butylamine, 1-ethyl-1-aminopropane, n-butylamine, n-propylamine.



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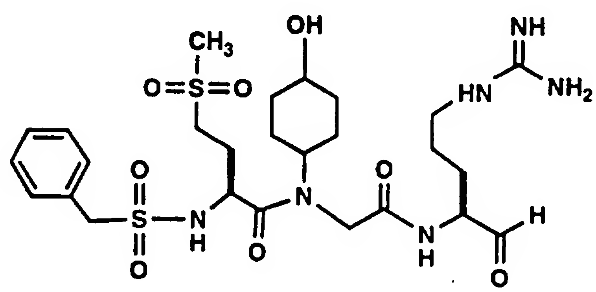
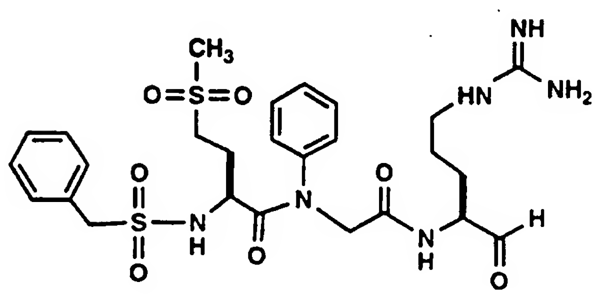
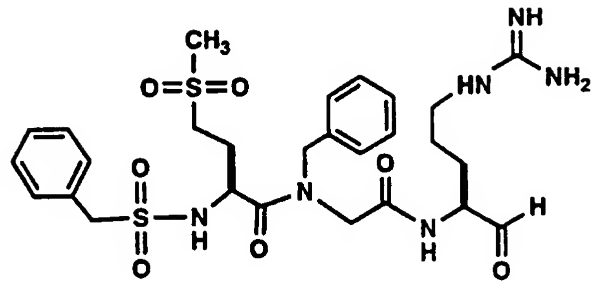


,

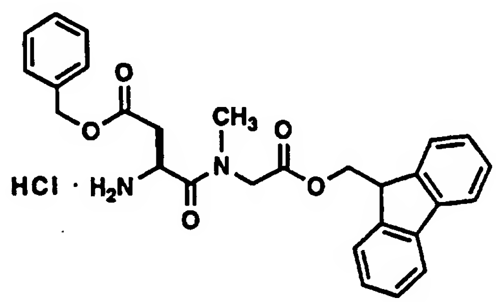


, and



Example 52

Preparation of aspartyl-beta-benzyl ester sarcosine-O-fluorenylmethyl ester, hydrochloride salt



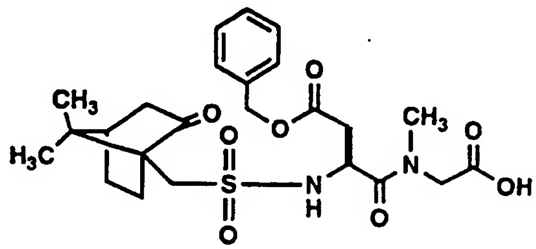
To a stirring solution of sarcosine-O-fluorenylmethyl ester, hydrochloric acid salt (5.6 g, 18.3 mmole) in 90 mL of dry dimethylformamide, was added N-Boc-aspartic acid beta-benzyl ester (6.5 g, 20.2 mmole) followed by benzotriazol-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (8.12 g, 18.4 mmole) and 2,4,6-collidine (5 mL, 36.7 mmole). The reaction mixture was stirred for 16 hours at room temperature, then was dissolved in 800 mL of ethyl acetate and washed with 200 mL each of water, 1 M aqueous hydrochloric acid, water, saturated aqueous sodium bicarbonate and brine. The organic phase was dried over anhydrous magnesium sulfate, filtered and the solvent removed *in vacuo*. Rf = 0.85 (1:9 methanol:dichloromethane).

The resulting oil was dissolved in dichloromethane (200 mL) and treated with 50 mL of 4.0M solution of hydrochloric acid in dioxane. After 12 hours, the title compound was precipitated by pouring the reaction mixture

into ether (500 mL) with vigorous stirring, filtered and dried *in vacuo* to provide 8.2 g (86% yield) of the title compound as an off-white powder. Rf = 0.5 (1:9 methanol:dichloromethane).

Example 53

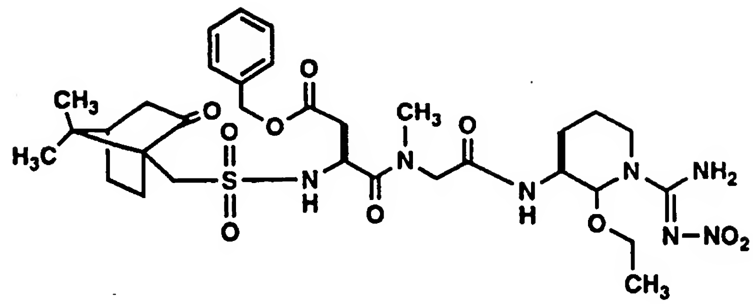
Preparation of (D)-camphorsulfonyl aspartyl-beta-benzyl ester sarcosine



To a solution of the compound of Example 52 (8.2 g, 15.7 mmole) in 30 mL of dry dimethylformamide and 50 mL acetonitrile was added (D)-camphorsulfonyl chloride (5.9 g, 23.6 mmole) followed by diisopropylethylamine (14 mL, 78.7 mmole). The reaction mixture was stirred for 16 hours at room temperature, at which time piperidine (10 mL, 100 mmole) was added to remove the fluorenylmethyl ester. After another 12 hours, the reaction mixture was dissolved in 600 mL of ethyl acetate and extracted in to saturated aqueous bicarbonate (2x200 mL). The combined aqueous fractions were washed with 200 mL ethyl acetate and then acidified to about pH 4 using hydrochloric acid. This was then extracted with ethyl acetate (2x300 mL) and the organic fractions were washed with 300 mL brine, dried over anhydrous magnesium sulfate, filtered and the solvent removed *in vacuo* to provide 6 g (55% yield) of the title compound as an off-white foam. Rf = 0.38 (1:9 methanol:dichloromethane).

Example 54

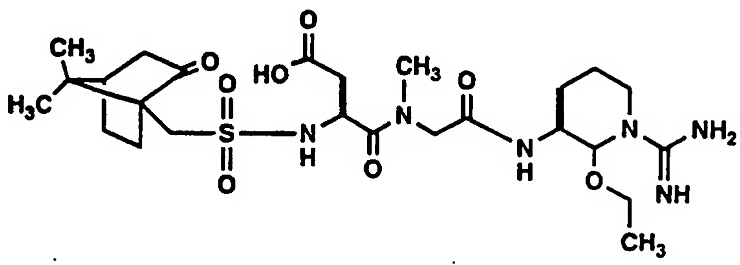
Preparation of (D)-camphorsulfonyl aspartyl-beta-benzyl ester sarcosine-N^g-NO_2-arginine cyclic-OEt aminal



The compound of Example 4 (1.18 g, 4.41 mmole) and the compound of Example 53 (2.1 g, 4.01 mmole) were dissolved with stirring in 10 mL of dry dimethylformamide and 30 mL of dry tetrahydrofuran. To this mixture was added 1-hydroxybenzotriazole monohydrate (0.81 g, 6.02 mmole) and 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (2.28 g, 6.02 mmole) followed by N-methylmorpholine (2 mL, 20 mmole). After 16 hours, the reaction mixture was diluted with 700 mL ethyl acetate and extracted with 150 mL each of water, 1 M aqueous hydrochloric acid, water, saturated aqueous sodium bicarbonate and brine. The organic phase was dried over anhydrous magnesium sulfate, filtered and the solvent removed *in vacuo* to afford 2.65 g (91% yield) of the title compound as an off-white foam. TLC gave two spots, Rf = 0.43 and 0.54 (1:9 methanol:dichloromethane).

Example 55

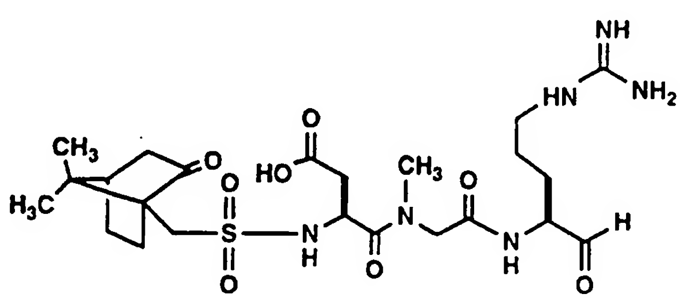
Preparation of (D)-camphorsulfonyl aspartyl sarcosine-arginine cyclic-OEt aminal



1 g of 10% palladium on carbon was placed in a 500 mL PARR bottle. 10 mL of water and 7 mL of glacial acetic acid was added. To this mixture was added a solution of the compound of Example 54 (2.6 g, 3.5 mmole) in 100 mL of methanol. The mixture was then shaken under a hydrogen atmosphere at 40 psi for 1 day. The catalyst was then removed by filtration and the filtrate concentrated *in vacuo*. The resulting oil was azeotroped with toluene to remove the remaining acetic acid to afford about 2 g of the title compound which was purified by HPLC on a two inch Vydak C-18 column at 115 mL/minute, gradient 20 to 60% over 50 minutes to give 0.7 g (30% yield) of the title compound as a white powder.

Example 56

Preparation of (D)-camphorsulfonyl aspartyl sarcosine arginine aldehyde

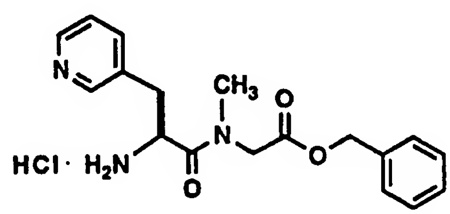


The compound of Example 55 (0.7 g, 1.2 mmole) was dissolved in 20 mL of 50:50 water:acetonitrile with

stirring and cooled to 0°C in an ice water bath. To this solution was slowly added 40 mL of a 60 wt% solution of hexafluorophosphoric acid in water. After 1.5 hour, the pH of the reaction mixture was adjusted to about pH 4 using saturated aqueous sodium acetate. This mixture was filtered through a plug of Celite. The title compound was obtained by purification from the filtrate by preparative HPLC (2 inch Vydak C18 column using a gradient consisting of 8 to 25% acetonitrile in water containing 0.1% trifluoroacetic acid run over 55 minutes at a flowrate of 115 mL/minute) and lyophilization of the pooled fractions. Mass spectroscopy (FAB) confirmed the theoretical molecular weight of 557.6.

Example 57

Preparation of 3-(3-pyridyl)-alanine sarcosine benzyl ester hydrochloride salt

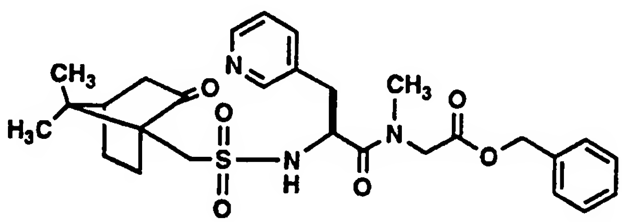


To a stirring solution of Boc-3-(3-pyridyl)-L-alanine (10 g, 37.5 mmole) in 190 mL of dry dimethylformamide was added sarcosine-O-benzyl ester, p-toluenesulfonic acid salt (13.2 g, 37.5 mmole) followed by benzotriazol-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (16.6 g, 37.5 mmole) and N-methylmorpholine (21 mL, 187.7 mmole). The mixture was stirred for 16 hours at room temperature. The reaction mixture was dissolved in 1000 mL of ethyl acetate and washed with 200 mL each of water, 1 M aqueous hydrochloric acid, water, saturated aqueous sodium bicarbonate and brine. The organic phase was dried over anhydrous magnesium sulfate, filtered and the solvent removed in vacuo to give an oil. Rf = 0.57 (1:9 methanol:dichloromethane).

The oil was dissolved in dichloromethane (250 mL) and treated with 50 mL of 4.0M solution of hydrochloric acid in dioxane. After 12 hours, the title compound was precipitated by pouring the reaction mixture into ether (500 mL) with vigorous stirring, filtered and dried in vacuo to provide 5.78 g (42% yield) of the title compound as an off-white powder.

Example 58

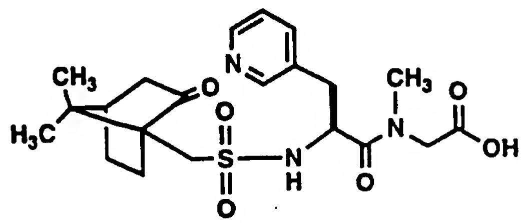
Preparation of (D)-camphorsulfonyl-3-(3-pyridyl)-alanine sarcosine benzyl ester



To a solution of the compound of Example 57 (3.3 g, 9.07 mmole) in 20 mL of dry dimethylformamide and 25 mL acetonitrile was added (D)-camphorsulfonyl chloride (3.4 g, 13.6 mmole) followed by triethylamine (6 mL, 45 mmole). The reaction mixture was stirred for 16 hours at room temperature. The reaction mixture was dissolved in 700 mL of ethyl acetate and washed with 100 mL each of water, saturated aqueous sodium bicarbonate and brine. The organic phase was dried over anhydrous magnesium sulfate, filtered and the solvent removed *in vacuo* to give an oil. The oil was dissolved in dichloromethane and filtered through silica, eluting first with dichloromethane (500 mL) then 1:9 methanol:dichloromethane (1000 mL). The methanol:dichloromethane fraction was concentrated to provide 3.33 g (68% yield) of the title compound as a brownish-yellow foam. Rf = 0.52 (1:9 methanol:dichloromethane).

Example 59

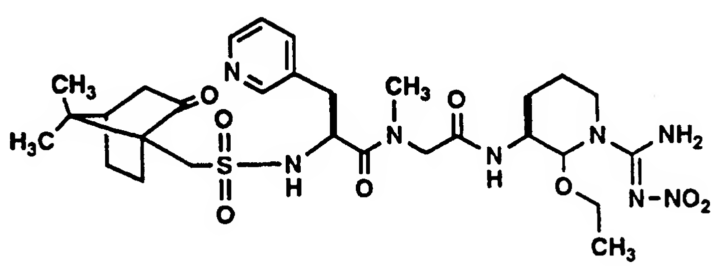
Preparation of (D)-camphorsulfonyl-3-(3-pyridyl)-alanine sarcosine



To a solution of the compound of Example 58 (3.33 g, 6.15 mmole) in 200 mL of methanol under a nitrogen blanket, was added 10% palladium on carbon (2.5 g) and the mixture was hydrogenated at 1 atmosphere for 4 days. The catalyst was removed and replaced 2 times during the course of the reaction. The mixture was then filtered and concentrated *in vacuo* to provide 2.0 g (72% yield) of the title compound as a white foam.

Example 60

Preparation of (D)-camphorsulfonyl-3-(3-pyridyl)-alanine sarcosine-N^g-NO_2-arginine cyclic-OEt aminal

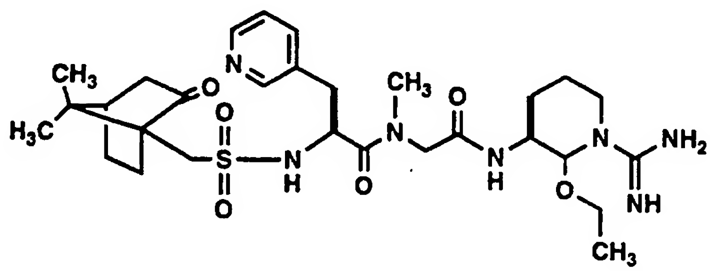


The compound of Example 4 (1.54 g, 5.76 mmole) and the compound of Example 59 (2 g, 4.4 mmole) were dissolved with stirring in 10 mL of dry dimethylformamide and 10 mL of dry acetonitrile. To this mixture was added 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride salt (1.3 g, 6.6 mmole) and 1-hydroxybenzotriazole monohydrate (0.9 g, 6.6 mmole) followed by diisopropylethylamine (3 mL, 22 mmole). After 16 hours, the reaction mixture was

concentrated *in vacuo* and then diluted with 500 mL ethyl acetate and extracted with 100 mL each of water, saturated aqueous sodium bicarbonate and brine. The organic phase was dried over anhydrous magnesium sulfate, filtered and the solvent removed *in vacuo* to afford 2.07 g (70% yield) of the title compound as an off-white foam. TLC gave two spots, Rf = 0.35 and 0.40 (1:9 methanol:dichloromethane).

Example 61

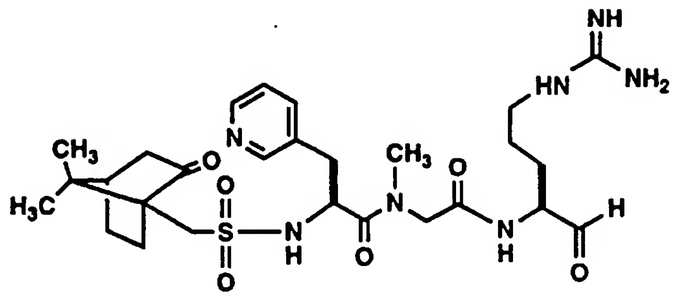
Preparation of (D)-camphorsulfonyl-3-(3-pyridyl)-alanine sarcosine arginine cyclic-OEt aminal



2 g of 10% palladium on carbon was placed in a 500 mL PARR bottle. 10 mL of water and 6 mL of glacial acetic acid was added. To this mixture was added a solution of the compound of Example 60 (2.07 g, 3.11 mmole) in 100 mL of methanol. The mixture was then shaken under a hydrogen atmosphere at 40 psi for 3 days. The catalyst was then removed by filtration and the filtrate concentrated *in vacuo* to give an oil. The oil was azeotroped with toluene to remove the remaining acetic acid to afford about 1.9 g (quantitative yield) of the title compound.

Example 62

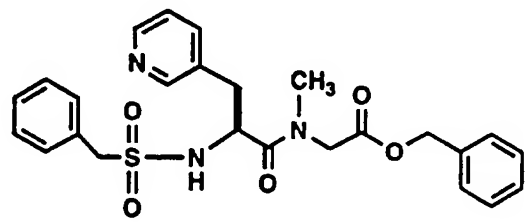
Preparation of (D)-camphorsulfonyl-3-(3-pyridyl)-alanine sarcosine arginine aldehyde



The compound of Example 61 (1.9 g, 3.1 mmole) was dissolved in 30 mL of 50:50 water:acetonitrile with stirring and cooled to 0°C in an ice water bath. To this solution was slowly added 40 mL of a 60 wt% solution of hexafluorophosphoric acid in water. After 2 hours, the pH of the reaction mixture was adjusted to about pH 4 using saturated aqueous sodium acetate. This mixture was filtered through a plug of Celite. The title compound was obtained by purification from the filtrate by preparative HPLC (2 inch Vydak C18 column using a gradient consisting of 8 to 25% acetonitrile in water containing 0.1% trifluoroacetic acid run over 55 minutes at a flowrate of 115 mL/minute) and lyophilization of the pooled fractions. Mass spectroscopy (FAB) confirmed the theoretical molecular weight of 591.7.

Example 63

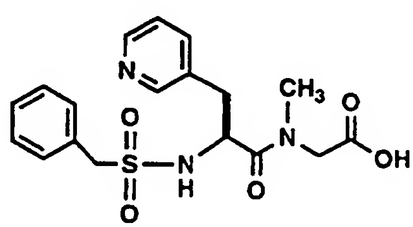
Preparation of benzylsulfonyl-3-(3-pyridyl)-alanine sarcosine benzyl ester



The title compound was prepared under the same manner as described in Example 58, using 2.44 g (6.7 mmole) of the compound of Example 57 in 10 mL of dry dimethylformamide and 25 mL acetonitrile, alpha-toluenesulfonyl chloride (1.9 g, 10.1 mmole), and triethylamine (5 mL, 34 mmole). This afforded 0.86 g (27% yield) of the title compound as a brownish-yellow foam. Rf = 0.45 (1:9 methanol:dichloromethane).

Example 64

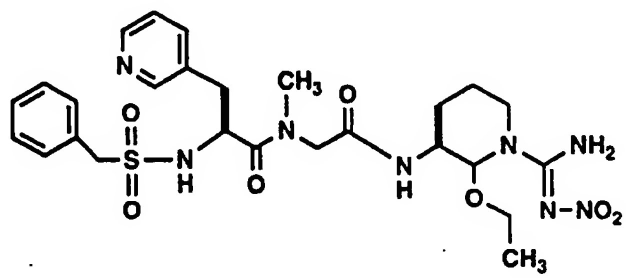
Preparation of benzylsulfonyl-3-(3-pyridyl)-alanine sarcosine



The title compound was prepared in the same manner as described in Example 59 using the compound of Example 63 (0.86 g, 1.78 mmole) in 100 mL of methanol and 0.5 g 10% palladium on carbon to provide 0.37 g (53% yield) of the title compound as a white foam.

Example 65

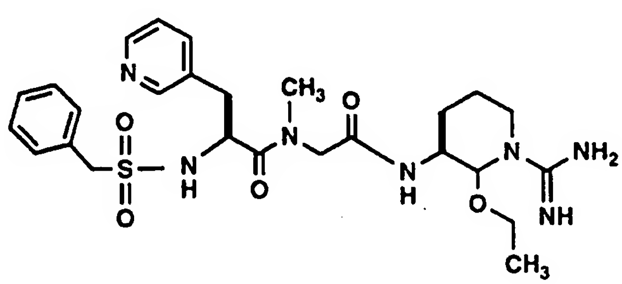
Preparation of benzylsulfonyl-3-(3-pyridyl)-alanine sarcosine N^g-NO_2-arginine cyclic-OEt aminal



The title compound was prepared in the same manner as described in Example 60 using the compound of Example 4 (0.33 g, 1.23 mmole), the compound of Example 64 (0.37 g, 0.95 mmole), 5 mL of dry dimethylformamide and 5 mL of dry acetonitrile, 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride salt (0.27 g, 1.4 mmole) and 1-hydroxybenzotriazole monohydrate (0.2 g, 1.4 mmole) and diisopropylethylamine (1 mL, 1.3 mmole) to afford 0.32 g (70% yield) of the title compound as an off-white foam. TLC gave two spots, Rf = 0.30 and 0.35 (1:9 methanol:dichloromethane).

Example 66

Preparation of benzylsulfonyl-3-(3-pyridyl)-alanine sarcosine arginine cyclic-OEt aminal

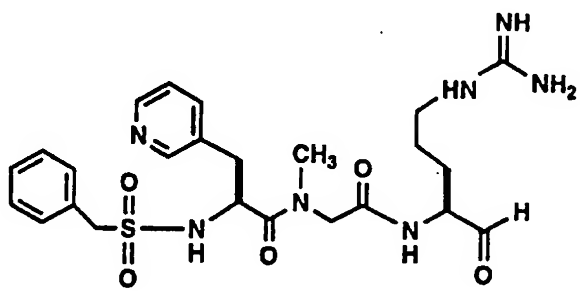


The title compound was prepared in the same manner as described in Example 61 using 0.3 g of 10% palladium on

carbon, 5 mL of water, 1 mL of glacial acetic acid, the compound of Example 65 (0.32 g, 0.53 mmole) in 30 mL of methanol to afford about 0.3 g (quantitative yield) of the title compound.

Example 67

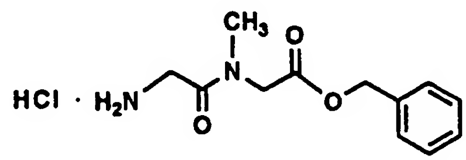
Preparation of benzylsulfonyl-3-(3-pyridyl)-alanine sarcosine arginine aldehyde



The title compound was prepared in the same manner as in Example 62 using the compound of Example 66 (0.29 g, 0.5 mmole), 10 mL of 50:50 water:acetonitrile and 20 mL of a 60 wt% solution of hexafluorophosphoric acid in water. After 4 hours, the pH of the reaction mixture was adjusted to about pH 4 using saturated aqueous sodium acetate. This mixture was filtered thru a plug of Celite. The title compound was obtained by purification from the filtrate by preparative HPLC (2 inch Vydak C18 column using a gradient consisting of 5 to 15% acetonitrile in water containing 0.1% trifluoroacetic acid run over 60 minutes at a flowrate of 115 mL/minute) and lyophilization of the pooled fractions. Mass spectroscopy (FAB) confirmed the theoretical molecular weight of 531.6.

Example 68

Preparation of glycine sarcosine benzyl ester hydrochloride salt

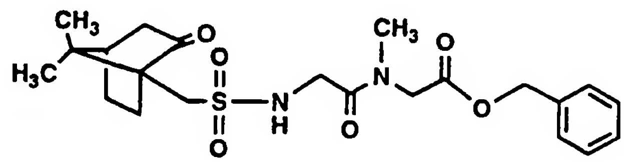


To a stirring solution of Boc-glycine (5.7 g, 32.5 mmole) in 160 mL of dry dimethylformamide, was added sarcosine benzyl ester p-toluenesulfonic acid salt (17.1 g, 48.8 mmole) followed by benzotriazol-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (14.4 g, 32.5 mmole) and N-methylmorpholine (18 mL, 162.7 mmole). The reaction mixture was stirred for 16 hours at room temperature. The reaction mixture was dissolved in 1000 mL of ethyl acetate and washed with 300 mL each of water, 1 M aqueous hydrochloric acid, water, saturated aqueous sodium bicarbonate and brine. The organic phase was dried over anhydrous magnesium sulfate, filtered and the solvent removed in vacuo. Rf = 0.78 (1:9 methanol : dichloromethane).

The resulting oil was dissolved in dichloromethane (100 mL) and then treated with 50 mL of 4.0M solution of hydrochloric acid in dioxane. After 5 hours, 8.8 g (quantitative yield) title compound was isolated as a off-white foam by removing the solvent *in vacuo* and azeotroping with toluene.

Example 69

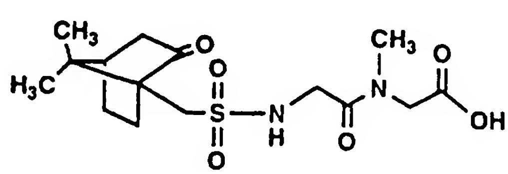
Preparation of (D)-camphorsulfonyl-glycine sarcosine benzyl ester



To a solution of the compound of Example 68 (8.8 g, 32.3 mmole) in 20 mL of dry dimethylformamide and 150 mL tetrahydrofuran was added (D)-camphorsulfonyl chloride (12.1 g, 48.4 mmole) followed by triethylamine (22 mL, 161 mmole). The mixture was stirred for 16 hours at room temperature. The reaction mixture was dissolved in 1000 mL of ethyl acetate and washed with 300 mL each of water, 1 M aqueous hydrochloric acid, water, saturated aqueous sodium bicarbonate and brine. The organic phase was dried over anhydrous magnesium sulfate, filtered and the solvent removed in vacuo to give an oil. The oil was dissolved in dichloromethane and filtered through silica, eluting first with dichloromethane (500 mL) then 1:9 methanol: dichloromethane (1000 mL). The methanol:dichloromethane fraction was concentrated to provide 13.37 g (91% yield) of the title compound as an off-white foam. Rf = 0.77 (1:9 methanol:dichloromethane).

Example 70

Preparation of (D)-camphorsulfonyl-glycine sarcosine

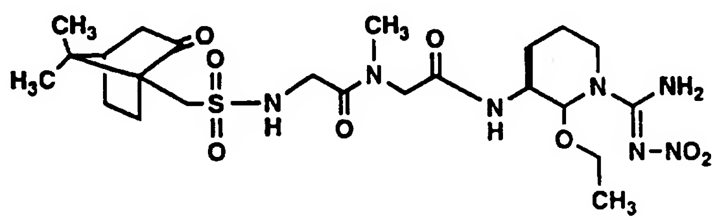


To a solution of the compound of Example 69 (13 g, 28.9 mmole) in 600 mL of methanol under a nitrogen blanket, was added 10% palladium on carbon (5 g) and the

mixture was hydrogenated at 1 atmosphere for 16 hours. The mixture was then filtered and concentrated *in vacuo* to give an oil. The oil was dissolved in 400 mL saturated aqueous sodium bicarbonate and washed with 300 mL ethyl acetate. The aqueous fraction was then acidified using hydrochloric acid to about pH 4 and extracted with ethyl acetate (2x500 mL). The combined organic fractions were washed with brine (2x300 mL), dried over anhydrous magnesium sulfate, filtered and concentrated *in vacuo* to provide 2.12 g (21% yield) of the title compound as a white foam. Rf = 0.31 (1:9 methanol:dichloromethane).

Example 71

Preparation of (D)-camphorsulfonyl-glycine sarcosine-N^g-NO_2-arginine cyclic-OEt aminal

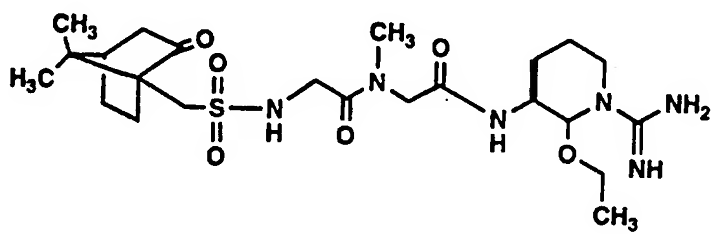


The compound of Example 4 (2.37 g, 8.85 mmole) and the the compound of Example 70 (2.12 g, 5.90 mmole) were dissolved with stirring in 8 mL of dry dimethylformamide and 20 mL of dry acetonitrile. To this mixture was added 1-hydroxy-7-azabenzotriazole (0.4 g, 2.95 mmole) and O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (2.2 g, 5.9 mmole) followed by N-methylmorpholine (3 mL, 29.5 mmole). After 16 hours, the reaction mixture was concentrated *in vacuo* and then diluted with 600 mL ethyl acetate and extracted with 200 mL each of water, 1 M aqueous hydrochloric acid, water, saturated aqueous sodium bicarbonate and brine. The organic phase was dried over anhydrous magnesium sulfate, filtered and the solvent removed *in vacuo* to give an oil. Chromatography of the oil on silica gel (eluting with 4:1:4 hexanes:methanol: dichloromethane) afforded 2.0 g

(59% yield) of the title compound as an off-white foam. TLC gave two spots, Rf = 0.36 and 0.44 (1:9 methanol:dichloromethane).

Example 72

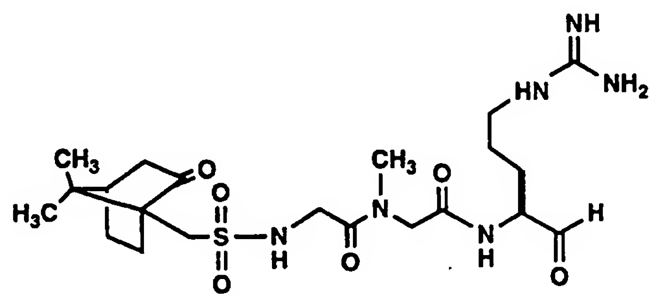
Preparation of (D)-camphorsulfonyl-glycine-sarcosine-arginine cyclic-OEt aminal



1 g of 10% palladium on carbon was placed in a 500 mL PARR bottle. 10 mL of water and 7 mL of glacial acetic acid was added. To this mixture was added a solution of the compound of Example 71 (2 g, 3.48 mmole) in 100 mL of methanol. The mixture was then shaken under a hydrogen atmosphere at 40 psi for 1 day. The catalyst was removed by filtration and the filtrate concentrated *in vacuo* to give an oil. 0.57 g (31% yield) title compound was obtained from this oil by purification by preparative HPLC (2 inch Vydak C18 column using a gradient consisting of 10 to 30% acetonitrile in water containing 0.1% trifluoroacetic acid run over 55 minutes at a flowrate of 115 mL/minute).

Example 73

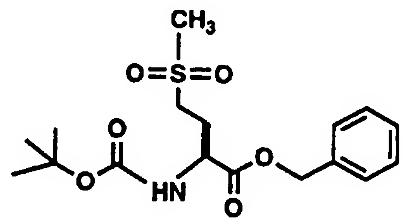
Preparation of (D)-camphorsulfonyl-glycine-sarcosine-arginine aldehyde



The compound of Example 72 (0.57 g, 1.08 mmole) was dissolved in 20 mL of 50:50 water:acetonitrile with stirring and cooled to 0°C in an ice water bath. To this solution was slowly added 30 mL of a 60 wt% solution of hexafluorophosphoric acid in water. After 1 hour, the pH of the reaction mixture was adjusted to about pH 4 using saturated aqueous sodium acetate. This mixture was filtered thru a plug of Celite. The title compound was obtained by purification from the filtrate by preparative HPLC (2 inch Vydak C18 column using a gradient consisting of 7-27% acetonitrile in water containing 0.1% trifluoroacetic acid run over 60 minutes at a flowrate of 115 mL/minute) and lyophilization of the pooled fractions. Mass spectroscopy (FAB) confirmed the theoretical molecular weight of 500.6.

Example 74a

Preparation of N-Boc-L-methionine sulfone-O-benzyl ester



To a solution of N-Boc-L-methionine sulfone (50 g, 178 mmole) in dry THF (500 mL) which had been chilled to 0°C, carbonyl diimidazole (34.6 g, 214 mmole) was added in small portions. After 30 minutes, the mixture was warmed to room temperature for 2 hours until all of the CO_2 evolution ceased. After this time, benzyl alcohol (27.6 mL, 267 mmole) was added and the reaction stirred for 12 hours.

The reaction mixture was then reduced in volume under vacuum and the resulting residue was diluted with ethyl acetate (500 mL). The organic phase was then washed with saturated bicarbonate (1x100 mL), brine (100 mL), then saturated aqueous citric acid (1x100 mL), dried over $MgSO_4$, filtered and the solvent removed under vacuum to provide a white solid. The white solid was washed with a 1:1 mixture of diethyl ether/hexanes (300 mL) and filtered off on a Büchner funnel to provide 50.0g (92%) of the title compound. Thin layer chromatography analysis of the title compound showed a single spot with R_f=0.18 (silica, 3:2 hexanes/ethyl acetate).

Example 74b

Preparation of N-Boc-N-phenethyl-L-methionine sulfone sarcosine benzyl ester

To a solution of N-Boc-L-methionine sulfone sarcosine benzyl ester, Example 74a, (4.7 g, 10.0 mmole) in dry N,N-dimethyl formamide (20 mL) at 0°C, is added (2-iodoethyl)benzene (2.9 mL, 20.0 mmole) followed by sodium hydride (60% dispersion, 400 mg, 10.0 mmole). The reaction is allowed to warm to room temperature and stirred for 24 hours. The reaction is diluted with ethyl acetate (200 mL) and washed successively with saturated sodium bicarbonate (1x75 mL), brine (1x75 mL) and 1 M aqueous hydrochloric acid (1x75 mL). The organic phase is dried over $MgSO_4$, filtered and the solvent removed in vacuo to provide the crude alkylated material. This material is purified on a flush silica gel column to give purified material.

Example 75

Preparation of N-Boc-N-phenethyl-L-methionine sulfone sarcosine acid

To a solution of Example 74 (5.7 g, 10.0 mmole) in methanol (200 mL) is added 1.0 g 10% palladium on carbon and the reaction subjected to atmospheric hydrogenation for 24 hours. The reaction mixture is then filtered through a short plug of celite and the reaction volume reduced in vacuo to provide the desired acid.

Example 76

Preparation of N-Boc-N-phenethyl-L-methionine sulfone sarcosine cyclic nitro arginine ethyl aminal

To a solution of Example 75 (4.8 g, 10.0 mmole) in N,N-dimethyl formamide (25 mL), is added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (1.9 g, 10.0 mmole) followed by 1-hydroxybenzotriazole (2.3 g, 15.0 mmole) and the reaction stirred for 30 minutes. Then cyclic nitroarginine ethyl aminal hydrochloride (4.0 g, 15 mmole) is added followed by N-methyl morpholine (2.2 ml, 20.0 mmole). The reaction is then stirred at room temperature for 18 hours. The reaction mixture is diluted with ethyl acetate (300 mL) and washed successively with saturated sodium bicarbonate (1x100 mL), brine (1x100 mL) and 1 M aqueous hydrochloric acid (1x100 mL). The organic phase is dried over $MgSO_4$, filtered and the solvent removed *in vacuo* to provide the desired coupled product.

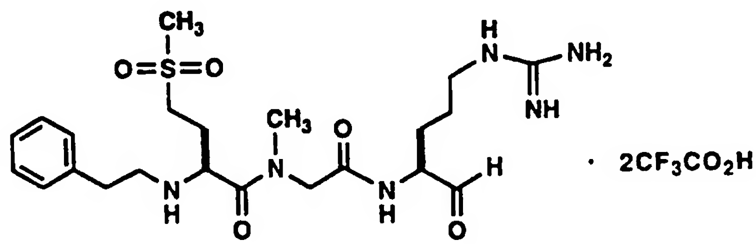
Example 77

Preparation of N-Boc-N-phenethyl-L-methionine sulfone sarcosine cyclic arginine ethyl aminal acetate salt

To a solution of Example 76 (6.9 g, 10.0 mmole) in water (60 mL), acetic acid (20 mL) and methanol (600 mL) in a 2000 mL Parr bottle is added 5.0 g of 10% palladium on carbon. The mixture is then shaken under a hydrogen atmosphere of 40 psi for 3 days. The catalyst is then removed by filtration and the filtrate concentrated *in vacuo*. The product is azeotroped with toluene to remove residual acetic acid to afford the title compound.

Example 78

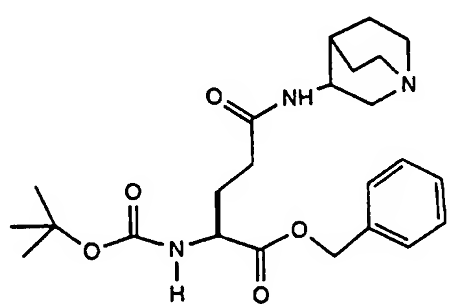
Preparation of N-phenethyl-L-methionine sulfone sarcosine argininal bistrifluoroacetate salt



The compound of Example 77 (6.4 g, 10.0 mmole) is dissolved in 200 mL of 1:1 water:acetonitrile with stirring and cooled to 0°C in an ice water bath. To this solution is slowly added 300 mL of a 60 wt% solution of hexafluorophosphoric acid in water. After about 1 hour, analytical HPLC (20 to 60% acetonitrile/water containing 0.1% trifluoroacetic acid) will indicate complete hydrolysis of the starting compound. The pH of the reaction mixture is adjusted to pH=4 using 3.0M aqueous sodium acetate. This mixture is filtered through a short plug of celite and then purified by preparative HPLC to provide the title compound.

Example 79

Preparation of N-Boc-L-Glutamate-(beta-3-(S)-amino quinuclidinyl)-alpha benzyl ester

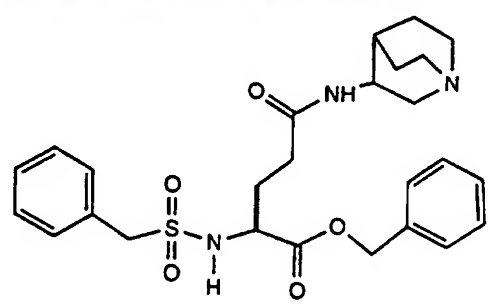


To a solution of N-Boc-L-glutamic acid-(beta-acid)-benzyl ester (3.3 g, 10.0 mmole) in N,N-dimethyl formamide

(50 mL), is added 1-hydroxy-7-azabenzotriazole (2.0 g, 15.0 mmole) and O-(7-azabenzotriazol-1-yl)-1,1,3,3,-tetramethylaronium hexafluorophosphate (3.8 g, 10.0 mmole) and the reaction stirred at room temperature for 30 minutes. Then 3-(R)-aminoquinuclidine dihydrochloride (3.0 g, 15.0 mmole) is added followed by N,N-diisopropylethyl amine (10.5 mL, 60.0 mmole) and the reaction stirred at room temperature for 18 hours. The reaction mixture is diluted with ethyl acetate (500 mL) and washed successively with saturated sodium bicarbonate (2x100 mL), water (2x100 mL) and brine (2x100 mL). The organic phase is dried over $MgSO_4$, filtered and the solvent removed in vacuo to provide the title compound.

Example 80

Preparation of N-benzylsulfonyl-L-glutamate (beta-3(S)-amino quinuclidinyl)-alpha benzyl ester

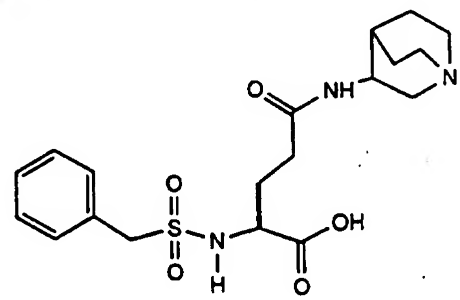


To a solution of Example 79 (4.5 g, 10.0 mmole) in dry ethyl acetate (100 mL) is added 4 M hydrochloric acid in dry dioxane (100 mL) at room temperature. The mixture is stirred for 3 hours and then evaporated *in vacuo* to provide the crude dihydrochloride salt. This compound is then dissolved in dry N,N-dimethyl formamide (50 mL) and benzylsulfonyl chloride (2.1 g, 12.0 mmole) is added followed by triethylamine (7.0 mL, 50.0 mmole). The reaction mixture is stirred at room temperature for 15 hours and then the reaction is diluted with ethyl acetate (400 mL). The organic phase is washed successively with saturated aqueous sodium bicarbonate (2x100 mL), water (2x100 mL) then brine (2x100 mL). The organic phase is

dried over $MgSO_4$, filtered and the solvent removed *in vacuo* to provide the title compound.

Example 81

Preparation of N-benzylsulfonyl-L-glutamic alpha acid-(beta-3(S)-amino quinuclidinyl)



To a solution of Example 80 (4.0 g, 10.0 mmole) in methanol (200 mL) is added 1.0 g of 10% palladium on carbon and the suspension subjected to atmospheric hydrogenation for 10 hours. The reaction mixture is filtered through a short plug of celite and the solvent is removed *in vacuo* to provide the title compound as the free acid.

Example 82

Preparation of N-benzylsulfonyl-L-glutamate-(beta-3(S)-amino quinuclidinyl)-sarcosine benzyl ester

To a solution of Example 81 (3.1 g, 10.0 mmole) in N,N-dimethyl formamide (40 mL) is added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (2.1 g, 11.0 mmole) and 1-hydroxybenzotriazole (2.3 g, 15.0 mmole). The mixture is stirred for 15 minutes at room temperature, then sarcosine benzyl ester p-toluenesulfonate salt (3.5 g, 10.0 mmole) is added, followed by N-methyl morpholine (2.2 mL, 20.0 mmole). The reaction is stirred for 15 hours, then diluted with ethyl acetate (300 mL) and washed successively with saturated sodium bicarbonate (2x100 mL), water (2x100 mL) and brine

(2x100 mL). The organic phase is dried over $MgSO_4$, filtered and the solvent removed *in vacuo* to provide the title compound.

Example 83

Preparation of N-benzylsulfonyl-L-glutamate-(beta-3(S)-amino quinuclidinyl)-sarcosine acid

To a solution of Example 82 (4.7 g, 10.0 mmole) in methanol (200 mL) is added 1.0 g 10% palladium on carbon and the reaction is subjected to atmospheric hydrogenation for 12 hours. The reaction is filtered through a short plug of celite and the solvent is removed *in vacuo* to provide the desired title compound.

Example 84

Preparation of N-benzylsulfonyl-L-glutamate-(beta-3(S)-amino quinuclidinyl)-sarcosine-cyclic nitro arginine ethyl aminal

The compound of Example 83 (3.8 g, 10.0 mmole) is dissolved in N,N-dimethyl formamide (30 mL) and 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (4.2 g, 11.0 mmole) is added, followed by 1-hydroxybenzotriazole monohydrate (2.3 g, 15.0 mmole). The reaction mixture is stirred for 15 minutes, then the cyclic nitro arginine ethyl aminal hydrochloride salt (3.7 g, 10.0 mmole) is added, followed by N-methyl morpholine (2.2 mL, 20.0 mmole) and the reaction mixture stirred for 12 hours. The reaction mixture is diluted with ethyl acetate (300 mL) and washed successively with saturated sodium bicarbonate (2x100 mL), water (2x100 mL) and brine (2x100 mL). The organic phase is dried over $MgSO_4$, filtered and the solvent removed *in vacuo* to provide the crude title compound.

Example 85

Preparation of N-benzylsulfonyl-L-glutamate-(beta-3(S)-amino quinuclidinyl-N'-(3-(1-propenyl))iodide)-sarcosine-cyclic nitro arginine ethyl aminal

To a solution of Example 84 (7.0 g, 10.0 mmole) in acetonitrile (25 mL) is added allyl iodide (1.8 mL, 20.0 mmole) and the reaction stirred at room temperature for 15 hours. The reaction is diluted with ethyl ether (250 mL) and the precipitate obtained is filtered off and dried *in vacuo* to provide the title compound.

Example 86

Preparation of N-benzylsulfonyl-L-glutamate-(beta-3(S)-amino quinuclidinyl-N'-propyl iodide salt)-sarcosine-cyclic arginine ethyl aminal

To a solution of Example 85 (8.6 g, 10.0 mmole) in water (60 mL), acetic acid (20 mL) and methanol (600 mL) in a 2000 mL Parr bottle is added 5.0 g of 10% palladium on carbon. The mixture is then shaken under a hydrogen atmosphere of 40 psi for 3 days. The catalyst is then removed by filtration and the filtrate concentrated *in vacuo*. The product is azeotroped with toluene to remove residual acetic acid to afford the title compound.

Example 87

Preparation of N-benzylsulfonyl-L-glutamate-(beta-3(S)-amino quinuclidinyl-N'-propyl iodide salt)-sarcosine-argininal

The compound of Example 86 (8.1 g, 10.0 mmole) is dissolved in 200 mL of 1:1 water:acetonitrile with stirring and cooled to 0°C in an ice water bath. To this solution is slowly added 300 mL of a 60 wt% solution of hexafluorophosphoric acid in water. After 1 hour analytical HPLC (20 to 60% acetonitrile/water containing 0.1% trifluoroacetic acid) indicated complete hydrolysis of the starting compound. The pH of the reaction mixture is adjusted to pH=4 using 3.0 M aqueous sodium acetate. This mixture is filtered through a short plug of celite and is then purified by preparative HPLC to provide the title compound.

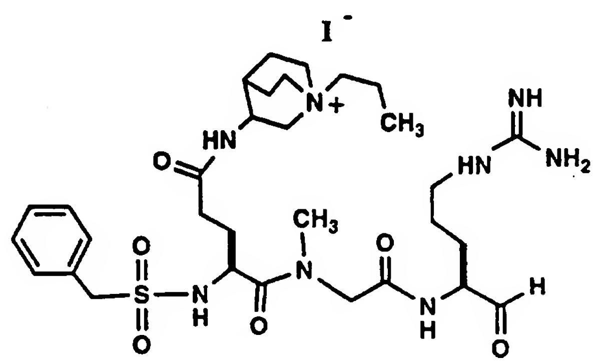
Example 88

Preparation of N-benzylsulfonyl-L-glutamate-(beta-3(S)-amino quinuclidinyl)-sarcosine-cyclic arginine ethyl aminal

To a solution of Example 87 (6.9 g, 10.0 mmole) in water (60 mL), acetic acid (20 mL) and methanol (600 mL) in a 2000 mL Parr bottle, is added 5.0 g of 10% palladium on carbon. The mixture is then shaken under a hydrogen atmosphere of 40 psi for 3 days. The catalyst is then removed by filtration and the filtrate concentrated *in vacuo*. The product is azeotroped with toluene to remove residual acetic acid to afford the title compound.

Example 89

Preparation of N-benzylsulfonyl-L-glutamate-(beta-3(S)-amino quinuclidinyl N'-propyl iodide salt)-sarcosine argininal



The compound of Example 88 (6.4 g, 10.0 mmole) is dissolved in 200 mL of 1:1 water:acetonitrile with stirring and cooled to 0°C in an ice water bath. To this solution is slowly added 300 mL of a 60 wt% solution of hexafluorophosphoric acid in water. After 1 hour analytical HPLC (20-60% acetonitrile/water containing 0.1% trifluoroacetic acid) indicates complete hydrolysis of the starting compound. The pH of the reaction mixture is adjusted to pH=4 using 3.0 M aqueous sodium acetate. This mixture is filtered through a short plug of celite and

then purified by preparative HPLC to provide the title compound.

Example 90

Preparation of N-benzylsulfonyl-S-(t-butylacetate)-L-cysteine sulfone-sarcosine-benzyl ester

A solution of the compound of Example 39 (5.0 g, 9.7 mmole) in 100 mL of sieve dried ethyl acetate is prepared. To this, 26 mL of 5.7 M anhydrous hydrochloric acid/ethyl acetate (that is generated *in situ* from acetyl chloride and dry methanol) is added dropwise. This mixture is stirred at room temperature for 8 hours until all starting material is consumed. The mixture is evaporated *in vacuo* and then azeotroped with toluene (3x50 mL). The resulting oil is suspended in acetonitrile (35 mL), cooled to ice bath temperature, then benzylsulfonyl chloride (2.1 g, 11.1 mmole) and pyridine (2.9 g, 37.1 mmole) are added. The reaction is removed from the ice bath after 30 minutes and allowed to stir at room temperature for 18 hours. The reaction mixture is reduced in volume *in vacuo*. The oil is taken up in 200 mL ethyl acetate and washed successively with 1 N hydrochloric acid (1x50 mL), saturated sodium bicarbonate (1x50 mL) and brine (1x50 mL). After drying with $MgSO_4$, the organic phase is reduced *in vacuo* to provide the desired sulfonamide product.

Example 91

Preparation of N-benzylsulfonyl-S-(carboxymethyl)-L-cysteine sulfone sarcosine benzyl ester

To a solution of the compound of Example 90 (5.5 g, 10.0 mmole) in dichloromethane (100 mL), is added trifluoroacetic acid (100 mL) and the mixture is stirred at room temperature for 4 hours, at which time starting material is consumed. The mixture is evaporated *in vacuo* to provide the desired acid.

Example 92

Preparation of N-benzylsulfonyl-S-((R)-alphamethyl/benzyl carboxymethyl amide)-L-cysteine sulfone sarcosine benzyl ester

To a solution of the compound of Example 91 (4.94 g, 10.0 mmole) in N,N-dimethyl formamide (40 mL), is added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (2.1 g, 11.0 mmole) and 1-hydroxybenzotriazole monohydrate (2.3 g, 15.0 mmole). The mixture is stirred for 15 minutes then (R)-alpha methyl benzyl amine (2.5 mL, 20.0 mmole) is added and the reaction stirred at room temperature for 12 hours. The reaction mixture is diluted with ethyl acetate (300 mL) and washed successively with saturated sodium bicarbonate (1x75 mL), brine (1x75 mL) and 1 M hydrochloric acid (1x75 mL). The organic phase is dried over $MgSO_4$, filtered and the volume reduced *in vacuo* to provide the desired carboxyamide.

Example 93

Preparation of N-benzylsulfonyl-S-((R)-alpha methyl benzyl carboxy methyl amide)-L-cysteine sulfone sarcosine acid

To a solution of the compound of Example 92 (5.97 g, 10.0 mmole) in methanol (100 mL), is added 1.0 g of 10% palladium on carbon and the mixture is stirred under hydrogen gas at atmospheric pressure for 24 hours. The mixture is then filtered through a short plug of celite and the volume is reduced *in vacuo* to provide the corresponding acid.

Example 94

Preparation of N-benzylsulfonyl-L-cysteine sulfone-S-((R)-alpha methyl benzyl carboxyamide)-sarcosine cyclic nitro arginine ethyl aminal

To a solution of Example 93 (5.1 g, 10.0 mmole) in N,N-dimethyl formamide (25 mL) is added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (1.9 g, 10.0 mmole), followed by 1-hydroxybenzotriazole monohydrate (2.3 g, 15.0 mmole), and then the reaction mixture is stirred for 30 minutes. Then, cyclic nitroarginine ethyl aminal hydrochloride (4.0 g, 15.0 mmole) is added, followed by N-methyl morpholine (2.2 ml, 20.0 mmole). The reaction mixture is then stirred at room temperature for 18 hours. The reaction mixture is diluted with ethyl acetate (300 mL) and washed successively with saturated sodium bicarbonate (1x100 mL), brine (1x100 mL) and 1 M aqueous hydrochloric acid (1x100 mL). The organic phase is dried over $MgSO_4$, filtered and the solvent removed *in vacuo* to provide the desired coupled product.

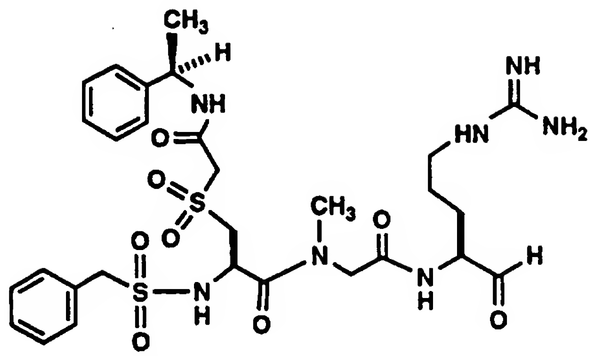
Example 95

Preparation of N-benzylsulfonyl-L-cysteinesulfone-S-((R)-alpha methyl benzyl carboxyamide)-sarcosine cyclic arginine ethyl aminal

To a solution of Example 94 (8.6 g, 10.0 mmole) in water (60 mL), acetic acid (20 mL) and methanol (600 mL) in a 2000 mL Parr bottle, is added 5.0 g of 10% palladium on carbon. The mixture is then shaken under a hydrogen atmosphere of 40 psi for 3 days. The catalyst is then removed by filtration and the filtrate concentrated *in vacuo*. The product is azeotroped with toluene to remove residual acetic acid to afford the title compound.

Example 96

Preparation of N-benzylsulfonyl-L-cysteinesulfone-S-((R)-alpha methyl benzyl carboxyamide)-sarcosine argininal

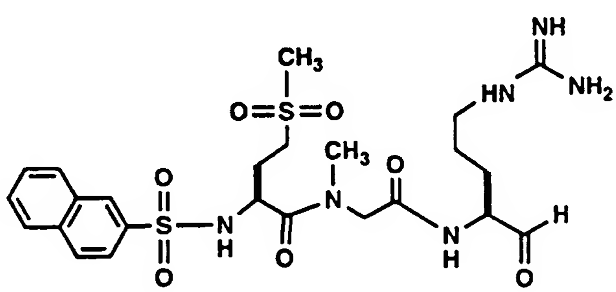


The compound of Example 95 (8.1 g, 10.0 mmole) is dissolved in 200 mL of 1:1 water:acetonitrile with stirring and cooled to 0°C in an ice water bath. To this solution is slowly added 300 mL of a 60 wt% solution of hexafluorophosphoric acid in water. After about 1 hour analytical HPLC (20 to 60% acetonitrile/water containing 0.1% trifluoroacetic acid) will indicate complete hydrolysis of the starting compound. The pH of the reaction mixture is adjusted to pH=4 using 3.0 M aqueous sodium acetate. This mixture is filtered through a short plug of celite and is then purified by preparative HPLC to provide the title compound.

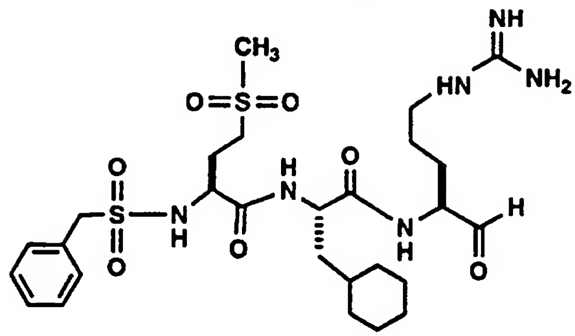
Example 97

Preparation of additional compounds

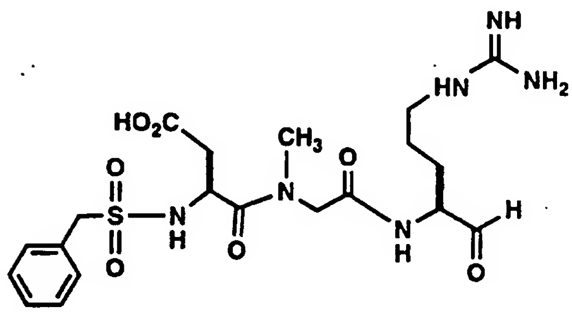
Using the methods described in Examples 1-96, the following additional compounds were synthesized:



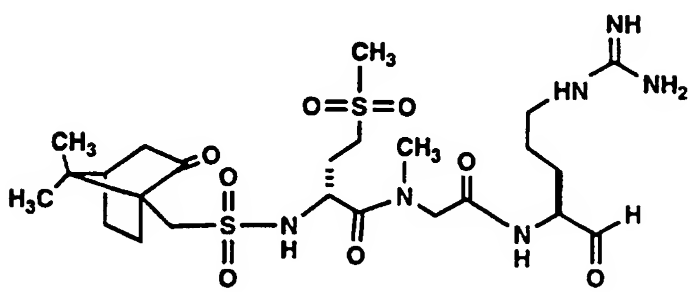
(97A),



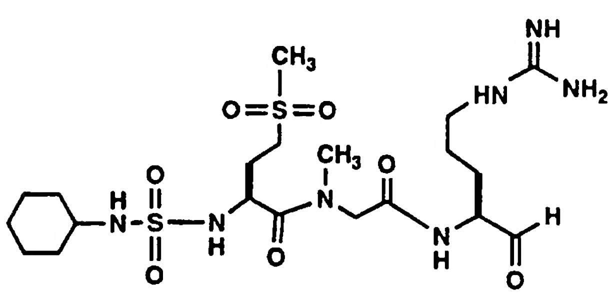
(97B),



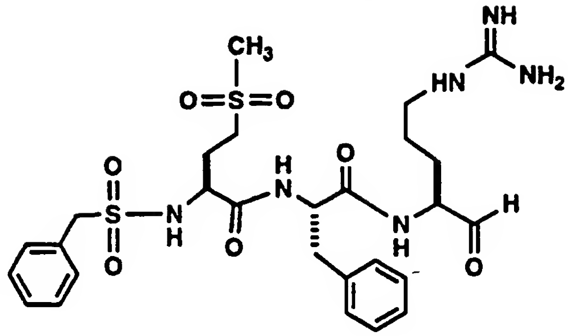
(97C),



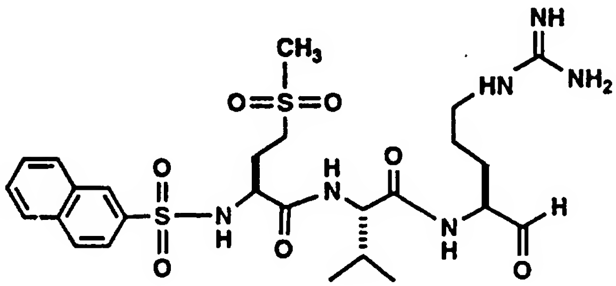
(97D),



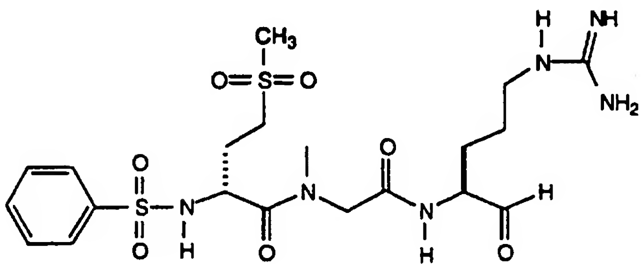
(97E),



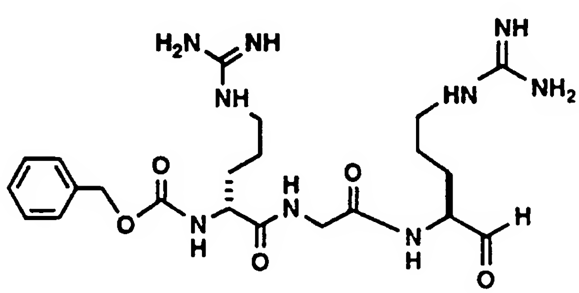
(97F),



(97G),



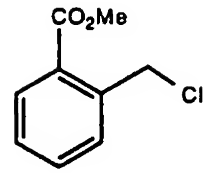
(97H), and



(97I).

Example 98

Preparation of methyl 2-chloromethyl benzoate

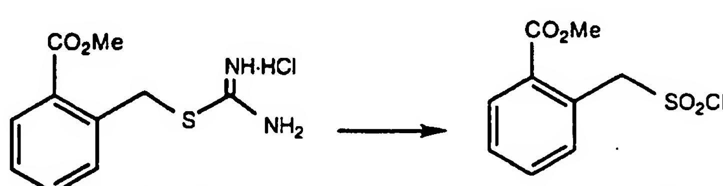


To 182 g (1.2 mole) of methyl 2-methyl benzoate and 3.6 g (0.022 mole) of 2,2'-azobis(2-methyl propionitrile (AIBN), at 60°C was added chlorine gas through a gas dispersion tube. The temperature was maintained at 64 to 66°C by the rate of the chlorine addition. The addition proceeded for 5 hours, nitrogen was passed through the mixture to remove most of the gases, then the mixture was filtered. The filtered material was dissolved in 500 mL of ether, the solution was washed twice with 200 mL of saturated $NaHCO_3$ and 200 mL of brine, dried over $MgSO_4$ and then evaporated *in vacuo* to yield a mixture of products (237 g). NMR($CDCl_3$) indicated the composition of product to be 65% desired monochloro product, 33% of dichloro product and 2% of starting material.

Note: It is helpful to monitor the reaction by NMR at 0.5 hour intervals and stop the chlorination when the monochloro product formation reaches 65 mole%. This keeps ring chlorination to a minimum (2nd type of benzyl protons in NMR). It appears that the maximum yield of the monochloro product is 65%.

Example 99

Preparation of 2-methoxycarbonyl benzyl sulfonyl chloride (via thiouronium salt)



Ex. 99A Ex. 99B

A. Preparation of Thiouronium Salt

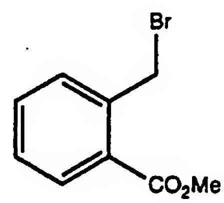
A mixture of the benzyl chlorid from Example 98 (142 g, 0.5 mole) and thiourea (38.1 g, 0.5 mol) in 500 mL of MeOH was refluxed for 4 hours, cooled, and the solvent was evaporated *in vacuo*. The residue was thoroughly triturated with ether and dried to yield 126 g of the thiouronium salt. NMR(DMSO-d_6) indicated 2 types of benzyl protons as well as the dichloromethyl byproduct generated in the preparation of the monochloro derivative.

B. Preparation of Sulfonyl Chloride

A mixture of thiouronium salt (63 g, Example 99A) in 1650 mL of water was mechanically stirred at ambient temperature for 30 minutes, cooled to 0° C and stirred for an additional 30 minutes. The mixture was filtered through a 0.2 micron nylon filter, keeping the mixture at 0° C throughout. (The filtered precipitate contained the dichloromethyl- and methyl methoxycarbonyl benzenes, byproduct and starting material from the preparation of the compound of Example 98). The aqueous filtrate was cooled to 0°C, and with mechanical stirring chlorine gas was bubbled through the solution. After the green color persisted, nitrogen was passed through the mixture to remove the excess chlorine, then 700 mL of ether was added and the layers were separated. The aqueous layer was again washed with 700 mL of ether. The combined ether solutions were in turn washed with 500 mL of 1% $NaHSO_3$, twice with 500 mL saturated $NaHCO_3$, 200 mL of brine, then dried over $MgSO_4$ and evaporated to yield 36.5 g of product. The crude sulfonyl chloride (90 g) was crystallized from 70 mL EtOAc. Product was filtered and washed with cold EtOAc to give 67 g of solid. An additional 10 g was obtained from the filtrate (yield for the 2 steps = 23.6%).

Example 100

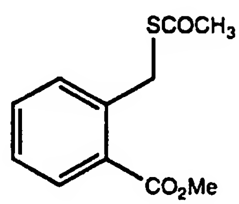
Preparation of Methyl 2-bromomethylbenzoate



To a mixture of methyl o-toluate (15.02 g, 0.10 mole, 14.0 mL) and NBS (19.58 g, 0.11 mol) in 200 mL of CCl_4, was added benzoyl peroxide (1.21 g, 0.0050 mole). The mixture was stirred, irradiated with a sunlamp and slowly heated to reflux. After 15 hours, the solution was cooled, filtered and evaporated. The residue was purified by flash chromatography on silica gel using a gradient system of hexane, methylene chloride: 20:1 to 2:1 and finally eluting with methylene chloride to afford 15.88 g (69% yield) of product as a pale yellow oil. TLC (silica gel; hexane, ethyl acetate): Rf= 0.3.

Example 101

Preparation of Methyl 2-acetylthiomethylbenzoate

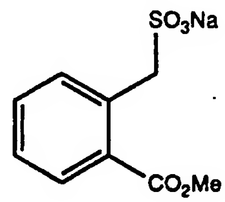


To a solution of the compound of Example 100 (15.87 g, 0.0693 mole) in 140 mL dry DMF at room temperatiure under N_2, was added potassium thioacetate (9.49 g, 0.083 mole, 1.2 equivalents) portionwise over about 20 minutes so as to maintain a temperature of 25 to 35C°. After 1 hour, the reaction mixture was poured into 500 mL of water with stirring. The mixture was extracted with 3 X 100 mL portions of ether. The combined organic phase was washed with 3 X 50 mL water, 1 X 50 mL brine, and then dried over

$MgSO_4$. Solvent removal afforded 14.88 g (96% crude yield) of product as a yellow oil, judged pure by TLC (silica gel; hexane, ether: 9, 1): Rf= 0.2.

Example 102

Preparation of o-Carbomethoxybenzylsulfonic acid, sodium salt

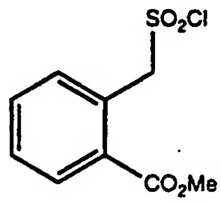


To a solution of the compound of Example 101 (14.88 g, 0.0664 mole) in 110 mL of acetic acid at 60°C, was added 30% H_2O_2 (41.4 mL, 0.332 mole) dropwise, carefully maintaining temperature at ~60 to 65°C. In spite of very slow addition and careful monitoring, after about 30 minutes addition time the reaction rapidly exothermed and refluxed at 108°C. After the exotherm subsided and the reaction cooled to 60°C, the remainder of the H_2O_2 was added over a one hour period. After 2 hours at 70 to 75°C, the reaction mixture was cooled to ambient temperature and stirred overnight. The solvents were azeotropically removed with heptane (water bath < 50°, SHIELD) and the residue was pumped *in vacuo* at < 1mm for 20 hours to afford 17.63 g (>Quantitative crude yield) of sulfonic acid as a viscous yellow oil. The oil was dissolved in 300 mL of water and neutralized carefully with ~75 mL of 1 N NaOH solution until the pH was 7. The solution was extracted with 3 X 100 mL portions of ether and briefly placed on the roto-vap to remove traces of ether. The water layer was lyophilized and afforded 17.45 g (104% yield) of product as a voluminous, colorless solid. TLC (silica gel; methylene chloride, methanol, acetic acid: 27, 3, 3): Rf= 0.2. NMR analysis showed the presence of ca 20% of the disodium salt resulting from ester hydrolysis. Subsequent runs on similar or larger

scales using either NaOH or $NaHCO_3$ for neutralization typically afforded product containing 13 to 20% disodium salt.

Example 103

Preparation of o-Carbomethoxybenzylsulfonyl chloride



To the compound of Example 102 (3.16 g @ 80%, 2.53 g, 0.010 mole) was added PCl_5 (2.08 g, 0.01 mole) and the resultant slurry was stirred and cooled in an ice bath while $POCl_3$ (11.50 g, 0.75 mole, 7.0 mL) was added slowly. The reaction mixture smoothly exothermed to 40°C and was allowed to stir at ambient temperature. After 15 hours, the mixture was heated to 40°C for 5 hours, cooled, diluted with acetonitrile, and then transferred to a one neck flask. The solvent and excess reagent were removed on the roto-vap. The crude residue was purified by rapid passage through a short silica gel column, eluting with hexane, ethyl acetate: 2, 1 to afford 2.00 g (80% yield) of product as a colorless, amorphous solid. TLC (silica gel; hexane, ethyl acetate: 2,1): Rf~ 0.5, noted a concentration-dependent streak.

Example 104

Preparation of (2-Carbomethoxy)benzyl-sulfonyl-D-N^g-NO_2-arginine sarcosine

The above-identified compound is prepared by coupling the product of either Example 99 or 103 with the product of Example 12 following procedures similar to those set forth in Example 13.

Example 105

Preparation of (2-carbomethoxy)benzylsulfonyl-D-N^g-NO_2-arginine-sarcosine-N^g-NO_2-arginine cyclic-OEt aminal

The HCl salt of the cyclicArg(NO_2)OEt aminal (0.56 g, 2.09 mmole) (compound of Example 4) and the (2-carbomethoxy)benzylsulfonyl-D-N^g-NO_2-arginine sarcosine (compound of Example 104) (1.05 g, 2.09 mmole) were dissolved with stirring in 10 mL of dry DMF. To this mixture was added EDC (0.60 g, 3.1 mmole) and HOBt (0.42 g, 3.1 mmole) followed by NMM (1.1 mL, 10.4 mmole). After 16 hours, the reaction mixture was diluted with 500 mL ethyl acetate and extracted with 100 mL each of water, 1 M aqueous HCl, water, saturated aqueous sodium bicarbonate and brine. The organic phase was dried over anhydrous magnesium sulfate, filtered and the solvent removed *in vacuo*. Chromatography of the resulting oil (silica, 1:9 methanol : methylene chloride) afforded 0.5 g (33% yield) of the title compound as an off-white foam. Rf = 0.39 (1:9 methanol : methylene chloride).

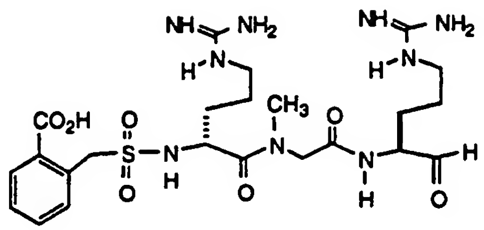
Example 106

Preparation of (2-carbomethoxy)benzylsulfonyl-D-arginine-sarcosine-arginine cyclic-OEt aminal

To the compound of Example 105 (0.5 g, 0.83 mmole) in 100 mL of 4:1 ethanol:acetic acid, was added 0.5 g moist Pearlman's catalyst. The mixture was then stirred vigorously under hydrogen at atmosphere pressure for 16 hours. The catalyst was then removed by filtration and the filtrate concentrated *in vacuo* to afford about 0.45 g (quantitative yield) of the title compound.

Example 107

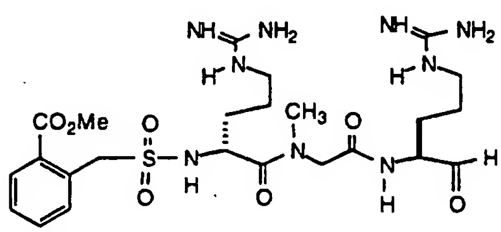
Preparation of (2-carboxy)benzylsulfonyl-D-arginine-sarcosine-arginine aldehyde



The compound of Example 106 (0.45 g, 0.83 mmole) was dissolved in 20 mL of 50:50 water:acetonitrile with stirring. To this solution was added 3 mL of 1M sodium hydroxide solution. After 16 hours, the reaction mixture was cooled in an ice water bath and 50 mL of a concentrated HCl was added. After 1.5 hour, the reaction was judged to be complete and the pH of the reaction mixture was adjusted to about pH=4 using saturated aqueous sodium acetate. This mixture was filtered and subsequent preparative HPLC afforded the title compound upon lyophilization of the pooled fractions.

Example 108

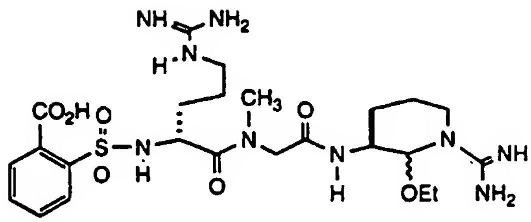
Preparation of (2-carbomethoxy)benzylsulfonyl-D-arginine-sarcosine-arginine aldehyde



The compound of Example 106 was hydrolyzed according to the procedure in Example 21 to afford the title compound.

Example 109

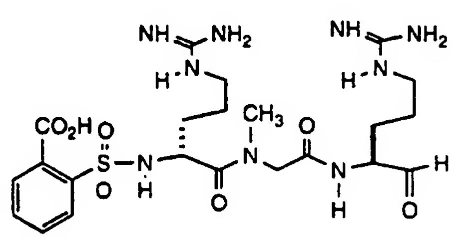
Preparation of (2-carboxy)benzenesulfonyl-D-arginine-sarcosine-arginine cyclic-OEt aminal



The Compound in Example 19 (0.6 g, 0.85 mmole) was dissolved in 40 mL of ethanol. To this was added 4 mL of 1M sodium hydroxide solution. After 4 hours, 60 mL of 4:1 ethanol:acetic acid was added, followed by 0.5 g of moist Pearlman's catalyst and the mixture was stirred under hydrogen at atmospheric pressure for 16 hours. The catalyst was then removed by filtration and the filtrate concentrated *in vacuo*. The mass spectrum showed both the title compound and some of the 2-carboethoxybenzenesulfonamide. The resulting oil was again dissolved in 20 ml of 50:50 water and acetonitrile with stirring. To this was added 2 mL of 1M sodium hydroxide solution. After 2 days, HPLC showed only one peak. The pH was neutralized using 1M HCl and the reaction mixture was concentrated *in vacuo* to give the above-identified product.

Example 110

Preparation of (2-carboxy)benzenesulfonyl-D-arginine-sarcosine-arginine aldehyde

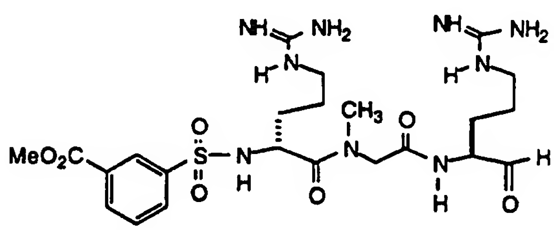


The compound of Example 109 (0.5 g, 0.8 mmole) was dissolved in 20 mL of 50:50 water and acetonitrile with stirring and cooled to 0°C in an ice water bath. To this solution was added 50 mL of concentrated HCl. After 5

hours, the reaction was judged complete by HPLC and the pH of the reaction mixture was adjusted to about pH=4 using saturated aqueous sodium acetate. This mixture was filtered and subsequent preparative HPLC afforded the title compound upon lyophilization of the pooled fractions.

Example 111

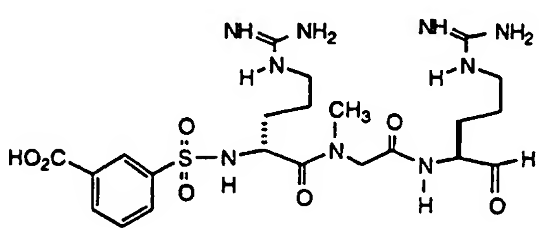
Preparation of (3 carbomethoxy)benzenesulfonyl-D-arginine-sarcosine-arginine aldehyde



The title compound was synthesized according to the procedures of Examples 17 to 21 using 3-carbomethoxy benzenesulfonyl chloride as the sulfonyl chloride in the procedures of Example 17.

Example 112

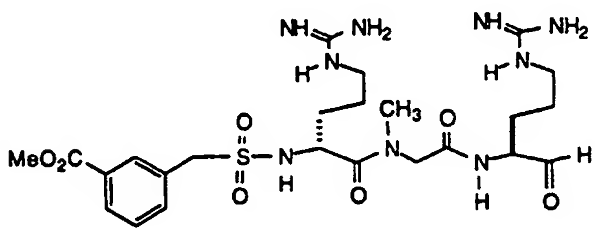
Preparation of (3-carboxy)benzylsulfonyl-D-arginine-sarcosine-arginine aldehyde



The title compound was synthesized according to the procedures of Examples 17 to 19, 109 and 110 using 3-carboxy-benzenesulfonyl chloride as the sulfonyl chloride in the procedure of Example 17.

Example 113

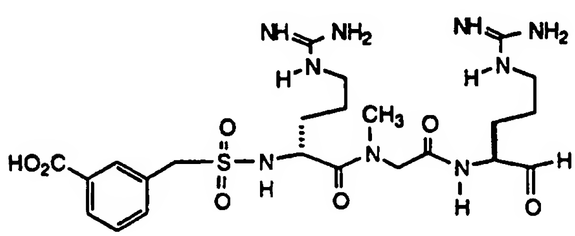
Preparation of (3-carbomethoxy)benzylsulfonyl-D-arginine-sarcosine-arginine aldehyde



The title compound was synthesized according to the procedures in Examples 105 to 108 using (3-carbomethoxy)benzylsulfonyl-D-N^g-NO_2-arginine sarcosine as a starting material in the procedure of Example 105.

Example 114

Preparation of (3-carboxy)benzylsulfonyl-D-arginine-sarcosine-arginine aldehyde



The title compound was synthesized according to the procedures in Examples 105 to 108 using (3-carboxy)benzylsulfonyl-D-N^g-NO_2-arginine sarcosine as a starting material in the procedure of Example 105.

Example 115

General Procedures for Preparing Compounds Having Camphor Derivatives at R_1

By following synthetic procedures such as those described in the Detailed Description of the Invention and in the Examples herein and using the appropriate reagents, the following compounds were made. Mass spectroscopy was used to confirm molecular weight.

Ex. No.	R_1	X	R_2	R_3	R_4	MS*
115A	d-Camphor	$-SO_2-$	$-CH_2COOH$	CH_3	H	557.6
115B	d-Camphor	$-SO_2-$	$-CH_2C(O)NH_2$	CH_3	H	558
115C	d-Camphor	$-SO_2-$	$-(CH_2)_2COOH$	CH_3	H	573
115D	d-Camphor	$-SO_2-$	$-(CH_2)_2C(O)OCH_3$	CH_3	H	587
115E	d-Camphor(OH)	$-SO_2-$	$-CH_2COOH$	CH_3	H	561

* Mass spectroscopy value

The above compounds were prepared using procedures similar to those set forth in Examples 5 to 10, 22 to 28, 36 to 44, 52 to 62 and/or 68 to 73, using the R_2 reagent noted in the Table below to supply the R_2 group which had the stereochemical configuration of the R_2 reagent.

R_2 Group	R_2 Reagent
$-(CH_2)_3NHC(=NH)NH_2$	Boc-D-N^g-nitroarginine
$-CH_2C(O)NH_2$	Boc-L-Asparagine Acid
$-CH_2COOH$	Boc-L-Aspartic Acid-ß-Benzyl ester
$-CH_2C(O)OCH_3$	Boc-L-Aspartic Acid-ß-Methyl ester
$-CH_2S(O)_2CH_2COOH$	L-Cysteine hydrochloride monohydrate
$-(CH_2)_2C(O)NH_2$	Boc-L-glutamine
$-(CH_2)_2COOH$	Boc-L-glutamic acid-ß-benzyl ester

$-(CH_2)_2C(O)OCH_3$	Boc-L-glutamic acid-ß-methyl ester
$-(CH_2)_2S(O)_2CH_3$	L-methionine sulfone-O-benzyl ester hydrochloride salt

Example 116

General Procedures For Preparation of Compounds Having Benzyl at R_1

The following compounds were prepared as set forth below. Mass spectroscopy was used to confirm molecular weights.

Ex. No.	R_1	X	R_2	R_3	R_4	MS*
116A	Benzyl	$-SO_2-$	$-(CH_2)_3NHC(=NH)NH_2$	CH_3	H	-
116B	Benzyl	$-SO_2-$	$-(CH_2)_2COOH$	CH_3	H	513
116C	Benzyl	$-SO_2-$	$-(CH_2)_2C(O)OCH_3$	CH_3	H	527
116D	Benzyl	$-SO_2-$	$-(CH_2)_2C(O)NH_2$	CH_3	H	512
116E	Benzyl	$-SO_2-$	$-CH_2C(O)NH_2$	CH_3	H	498
116F	Benzyl	$-SO_2-$	$-CH_2S(O)_2CH_2COOH$	CH_3	H	577
116G	Benzyl	$-SO_2-$	$-(CH_2)_3NHC(=NH)NH_2$+	CH_3	H	

* Mass spectroscopy value

\+ L-Configuration

The above noted compounds were prepared using procedures similar to those set forth in Examples 11 to 16, 29 to 35 and/or 63 to 67 using the R_2 reagent noted in the Table below to supply the R_2 group which has the stereochemical configuration noted for the R_2 reagent.

R_2 Group	R_2 Reagent
$-(CH_2)_3NHC(=NH)NH_2$	Boc-D-N^g-nitroarginine
$-CH_2C(O)NH_2$	Boc-L-Asparagine Acid
$-CH_2COOH$	Boc-L-Aspartic Acid-ß-Benzyl ester
$-CH_2C(O)OCH_3$	Boc-L-Aspartic Acid-ß-Methyl ester
$-CH_2S(O)_2CH_2COOH$	L-Cysteine hydrochloride monohydrate
$-(CH_2)_2C(O)NH_2$	Boc-L-glutamine
$-(CH_2)_2COOH$	Boc-L-glutamic acid-ß-benzyl ester
$-(CH_2)_2C(O)OCH_3$	Boc-L-glutamic acid-ß-methyl ester
$-(CH_2)_2S(O)_2CH_3$	Boc-L-methionine sulfone
$-(CH_2)_3NHC(=NH)NH_2+$	Boc-L-N^g-nitro arginine

Example 117

Preparation of Benzylsulfonyl-(D)-Arginine N-Methyl(O-Bn)-Arginine Aldehyde

The compound having the substituents noted below was prepared using procedures similar to those described in the Examples herein, using commercially available N-methyl-O-benzyl serine tert-butyl ester (in place of Boc-Sarcosine). Boc-N^g-D-Nitroarginine was used as the R_2 reagent. Mass spectroscopy was used to confirm molecular weight.

R_1	X	R_2	R_3	R_4	MS*
Benzyl	$-SO_2-$	$-(CH_2)_3NHC(=NH)NH_2$	CH_3	$-CH_2-OBn$	660

* Mass spectroscopy value

Example 118

General Procedures for Preparing Compounds Having Benzyl at R_1 and a L-Asp(OMe) Side Chain at R_2

The following compounds were prepared as set forth below. Mass spectroscopy was used to confirm molecular weight.

Ex. No.	R_1	X	R_2	R_3	R_4	MS*
118A	Benzyl	$-SO_2-$	$-CH_2C(O)OCH_3$	iso-propyl	H	541
118B	Benzyl	$-SO_2-$	$-CH_2C(O)OCH_3$	3-pentyl.	H	569
118C	Benzyl	$-SO_2-$	$-CH_2C(O)OCH_3$	cyclopentyl	H	567
118D	Benzyl	$-SO_2-$	$-CH_2C(O)OCH_3$	cyclohexyl	H	581
118E	Benzyl	$-SO_2-$	$-CH_2C(O)OCH_3$	i-butyl	H	555
118F	Benzyl	$-SO_2-$	$-CH_2C(O)OCH_3$	Ch_x-CH_2-	H	595
118G	Benzyl	$-SO_2-$	$-CH_2C(O)OCH_3$	benzyl	H	589
118H	Benzyl	$-SO_2-$	$-CH_2C(O)OCH_3$	n-propyl	H	541
118I	Benzyl	$-SO_2-$	$-CH_2C(O)OCH_3$	cyclobutyl	H	553

* Mass spectroscopy value

The above-noted compounds were prepared using procedures similar to those described in Examples 45 to 51, except that Boc-L-Aspartic Acid-ß-methyl ester was used as the R_2 reagent and the below noted R_3 reagents were used. The compounds had the same stereochemical configuration as the R_2 reagent.

R_3	R_3 **Reagent**
i-propyl	N-isopropyl-glycine-tert-butyl ester
3-pentyl	N-(3-pentyl)-glycine-tert-butyl ester
cyclopentyl-	N-cyclopentyl-glycine-tert-butyl ester
cyclohexyl	N-cyclohexyl-glycine-tert butyl ester
i-butyl	N-isobutyl glycine-tert butyl ester
Chx-CH_2	N-cyclohexylmethyl-tert butyl ester
benzyl	N-benzyl-glycine-tert butyl ester
n-propyl	N-propyl-glycine tert butyl ester
cyclobutyl	N-cyclobutyl-glycine tert butyl ester
Chx	N-cyclohexyl-glycine-tert-butyl ester
sec-butyl	N-sec-butyl-glycine-tert-butyl-ester

Example 119

General Procedures for Preparation of Compounds Having 4-tolyl Groups at R_1

The following compounds were prepared as set forth below. Mass spectroscopy was used to confirm molecular weight.

Ex. No.	R_1	X	R_2	R_3	R_4	MS*
119A	4-tolyl	-SO_2-	-$(CH_2)_3NHC(=NH)NH_2$	CH_3	H	540
119B	4-tolyl	-SO_2-	-$(CH_2)_2C(O)NH_2$	CH_3	H	512
119C	4-tolyl	-SO_2-	-$(CH_2)_2S(O)_2CH_3$	CH_3	H	547

* Mass spectroscopy value

The above noted compounds were prepared using procedures similar to those set forth in the Examples except that 4-toluene sulfonyl chloride was substituted for benzene sulfonyl chloride and the respective R_2 reagents noted below were used. The product conpounds had the same stereochemical configuration at R_2 as the R_2 reagent used.

R_2	R_2 R ag nt
$-(CH_2)_3NHC(=NH)NH_2$	Boc-D-N^g-nitroarginine
$-CH_2C(O)NH_2$	Boc-L-Asparagine acid
$-CH_2COOH$	Boc-L-Aspartic acid-ß-benzyl ester
$-CH_2C(O)OCH_3$	Boc-L-Aspartic acid-ß-methyl ester
$-CH_2S(O)_2CH_2COOH$	L-cysteine hydrochloride monohydrate
$-(CH_2)_2C(O)NH_2$	Boc-L-glutamine
$-(CH_2)_2C(O)OCH_3$	Boc-L-Glutamic acid-ß-benzyl ester
$-(CH_2)_2C(O)OCH_3$	Boc-L-Glutamic acid-ß-methyl ester
$-(CH_2)_2S(O)_2CH_3$	Boc-L-methionine sulfone

Example 120

General Procedures for Compounds Having D-Arg or L-Arg Side Chains at R_2

By following procedures similar to those set forth in the Examples 98 to 114 herein the following compounds were made:

Ex. No.	R_1	X	R_2	R_3	R_4	MS*
120A	Ph(2-CO_2Me)-	$-SO_2-$	$-(CH_2)_3NHC(=NH)NH_2$	methyl	H	584
120B	Ph(2-CO_2H)-	$-SO_2-$	$-(CH_2)_3NHC(=NH)NH_2$	methyl	H	570
120C	Ph(3-CO_2Me)-	$-SO_2-$	$-(CH_2)_3NHC(=NH)NH_2$	methyl	H	584
120D	Ph(3-CO_2H)-	$-SO_2-$	$-(CH_2)_3NHC(=NH)NH_2$	methyl	H	570
120E	Ph(2-CO_2Me)- CH_2-	$-SO_2-$	$-(CH_2)_3NHC(=NH)NH_2$	methyl	H	598
120F	Ph(2-CO_2H) CH_2-	$-SO_2-$	$-(CH_2)_3NHC(=NH)NH_2$	methyl	H	584
120G	Ph(3-CO_2Me)- CH_2-	$-SO_2-$	$-(CH_2)_3NHC(=NH)NH_2$	methyl	H	

120H	Ph(3-CO_2H)-CH_2-	-SO_2-	-$(CH_2)_3$NHC(=NH)NH_2	methyl	H	
120I	Ph(3-CO_2Me)	-SO_2-	-$(CH_2)_3$NHC(=NH)NH_2+	CH_3	H	
120J	Chx-CH_2-	-SO_2-	-$(CH_2)_3$NHC(=NH)NH_2	CH_3	H	
120K	Chx	-SO_2-	-$(CH_2)_3$NHC(=NH)NH_2	CH_3	H	

* Mass spectroscopy value

\+ L-Configuration

Example 121

General Procedure for Preparation of Compounds having I-Butyl at R_2

By following the procedures described herein, including Example 37 wherein methylchloroformate is substituted for di-t-butyl dicarbonate and D-leucine (R_2 reagent) is substituted for the compound of Example 36, such that these starting materials produce methoxycarbonyl-D-leucine for R_1-X-NH-CH(R_2)COOH, the following compounds were made. The resulting compounds had the same stereochemical configuration at R_2 as noted for the R_2 reagent.

Ex. No.	R_1	X	R_2	R_3	R_4	MS*
121A	methyl-O-	CO	i-butyl	methyl	-CH_2OH	431
121B	methyl-O-	CO	i-butyl	methyl	-CH$(CH_3)_2$	443

* Mass spectroscopy value

Example 122

General Procedure for Preparation of Compounds Having Alkyl Groups at R_1 and X as Carbonyl

By following the procedures described in the Detailed Description of the Invention and the Examples, including

using the R_3 reagents listed in Example 118 and using 2-propyl-pentanoic acid as starting material for R_1, the following compounds were made. Boc-L-aspartic acid-ß-methyl ester was used as the R_2 reagent. The product compounds had the same stereochemical configuratoin at R_2 as the R_2 reagent.

Ex. No.	R_1	X	R_2	R_3	R_4	MS*
122A	$(CH_3CH_2)_2CH-$	CO	$-CH_2C(O)OCH_3$	i-Propyl	H	513
122B	$(CH_3CH_2)_2CH-$	CO	$-CH_2C(O)OCH_3$	Chx-	H	553
122C	$(CH_3CH_2)_2CH-$	CO	$-CH_2C(O)OCH_3$	i-Butyl	H	527
122D	$(CH_3CH_2)_2CH-$	CO	$-CH_2C(O)OCH_3$	sec-Butyl	H	527

* Mass spectroscopy value

Example 123

Preparation of Compounds Having Menthyloxy Carbonyl at R_1-X

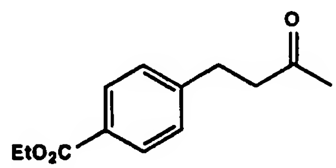
By following the procedures described in the Detailed Description of the Invention and the Examples herein, including using the R_2 reagents set forth in Example 115 to obtain the appropriate R_2 group and using menthyloxycarbonylchloride as starting material for the R_1-X group, the following compounds were made:

Ex. No.	R_1	X	R_2	R_3	R_4	MS*
123A	menthyl-O-	CO	L-$CysO_2$-(S--COOH)	methyl	H	681
123B	(-)menthyl-O-	CO	L-Glu(OMe)	methyl	H	
123C	(-)menthyl-O-	CO	L-Glu	methyl	H	
123D	(-)menthyl-O-	CO	L-Met(O_2)	methyl	H	

* Mass spectroscopy value

Example 124

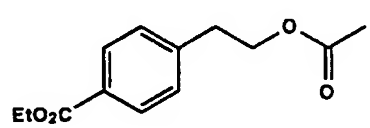
Preparation of 3-(4-carboethoxyphenyl)butan-2-one



Argon was bubbled through a suspension of ethyl 4-bromobenzoate (7.5 g), 3-buten-2-ol (3.6 g), triphenylphosphine (0.17 g), palladium acetate (0.073 g), and sodium bicarbonate (3.5 g) in DMF (40 mL) for 30 minutes. The reaction vessel was then placed in an oil bath at 100°C. After 3 days, the reaction mixture was cooled, filtered through celite, diluted with ethyl acetate (150 mL), washed with brine (2 X 300 mL), dried over magnesium sulfate, and concentrated. Silica gel chromatography using hexane and ethyl acetate (4:1) as eluent gave the titled compound (4.7 g) in 57% yield. Rf (Hexanes/EtOAc 1:1) was 0.59.

Example 125

Preparation of Ethyl 4-(2-acetoxyethyl)benzoate

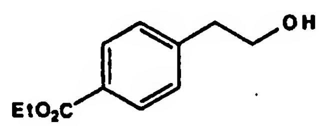


To a solution of the compound of Example 124 (4.4 g) in chloroform (100 mL) was added 50% m-chloroperbenzoic acid (13.8 g). The solution was refluxed for 19 hours, then an additional portion of 50% m-chloroperbenzoic acid (6.9 g) was added and reflux was continued for 24 hours. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The residue was diluted with ethyl acetate (150 mL), washed with sodium bicarbonate solution (100 mL), sodium thiosulfate solution (100 mL), and brine (100 mL), then

dried over magnesium sulfate and concentrated. Silica gel chromatography using hexane and ethyl acetate (gradient; 9:1 to 4:1) as eluent gave the titled compound (2.2 g) in 47% yield. Rf (Hexanes/EtOAc 1:1) was 0.70.

Example 126

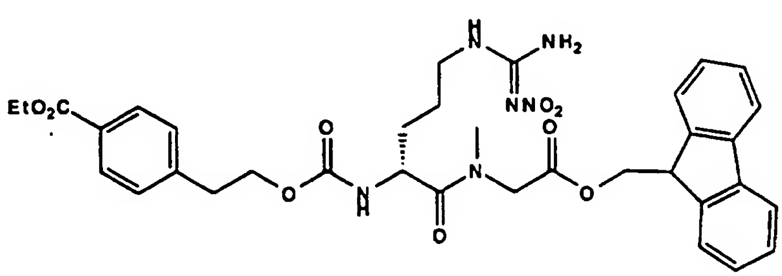
Preparation of Ethyl 4-(2-hydroxyethyl)benzoate



To a freshly prepared solution of sodium ethoxide (from 0.20 g sodium in 25 mL ethanol), was added the compound of Example 125 (2.1 g). The reaction mixture was heated in an oil bath at 65°C for 1 hour, then cooled to room temperature and neutralized with 3% hydrochloric acid. The solvent was then removed under reduced pressure. The residue was extracted into ethyl acetate (100 mL), washed with brine (50 mL), dried over magnesium sulfate, and concentrated. Silica gel chromatography using hexane and ethyl acetate (gradient; 9:1 to 2:1) as eluent gave the titled compound (1.2 g) in 70% yield. Rf (Hexanes/EtOAc 1:1) was 0.41.

Example 127

Preparation of 2-(4-carboethoxyphenyl)ethylcarbamoyl-(d)-N^g-NO_2-argininylsarcosine fluorenylmethyl ester

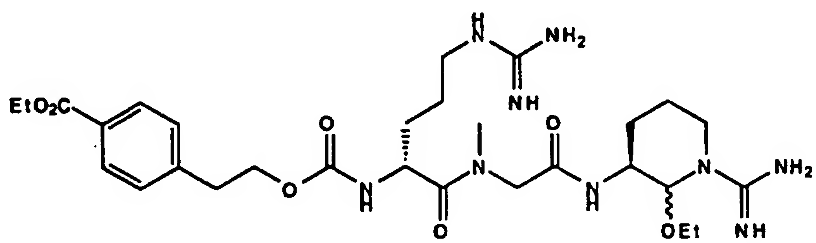


A solution of the compound of Example 126 (1.0 g) and collidine (2.7 mL) in dichloromethane (10 mL) was added

over 2 minutes to a solution of phosgene (2.7 mL; 1.93 M solution in toluene) in dichloromethane (15 mL) at 0°C. After 45 minutes, (d)-N^g-NO_2-argininylsarcosine fluorenylmethyl ester hydrochloride salt (2.6 g; product of Example 12) was added in one portion. The cooling bath was removed and the reaction mixture was stirred at room temperature for 22 hours. After neutralizing the mixture with 3% hydrochloric acid and concentrating under reduced pressure, the residue was diluted with ethyl acetate (200 mL), washed with 3% hydrochloric acid (50 mL), brine (50 mL), sodium bicarbonate solution (50 mL), dried over magnesium sulfate, and concentrated to give the titled compound (3.4 g) in 95% yield. Rf(CH_2Cl_2/MeOH 95:5) was 0.19.

Example 128

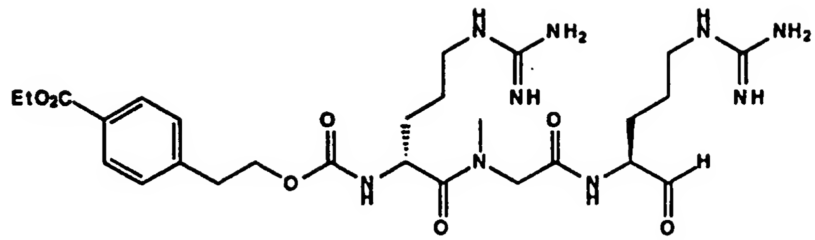
Preparation of 2-(4-carboethoxyphenyl)ethylcarbamoyl-(d)-argininylsarcosinylarginine ethyl aminal



The ester of Example 127 (3.4 g) was deblocked using piperidine as described in Example 18; the resulting carboxylic acid was coupled to N^g-NO_2arginine ethyl aminal hydrochloride salt as described in Example 19; and hydrogenolysis of the nitro groups as described in Example 20 gave the title compound (1.8 g) in 59% overall yield.

Example 129

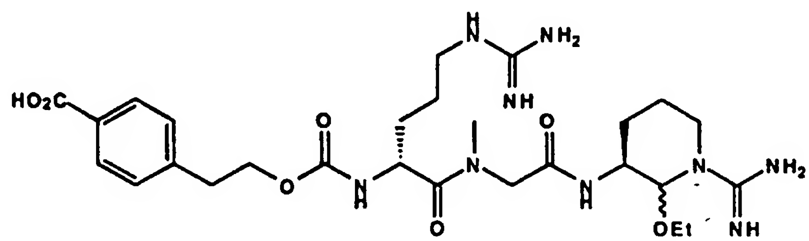
Preparation of 2-(4-carboethoxyphenyl)ethylcarbamoyl-(d)-argininylsarcosinylargininal



The compound of Example 128 (1.0 g) was hydrolyzed as described in Example 21 to give the title compound (0.54 g)

Example 130

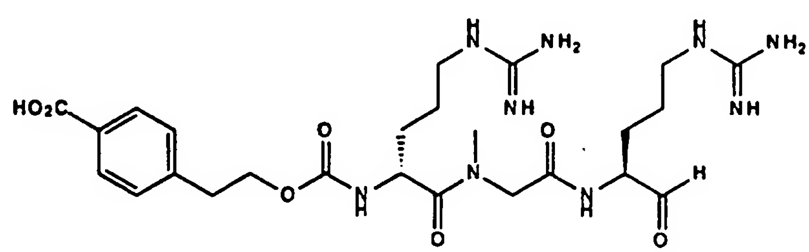
Preparation of 2-(4-carboxyphenyl)ethylcarbamoyl-(d)-argininylsarcosinylarginine ethyl aminal



The compound of Example 128 (1.8 g) was dissolved in ethanol (5.0 mL) and lithium hydroxide (6.0 mL) was added. The reaction mixture was stirred for 4 hours, then was neutralized with 3% HCl (to pH=6), and concentrated to give the title compound.

Example 131

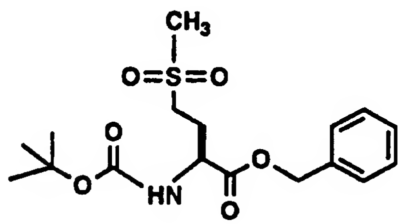
Preparation of 2-(4-carboxyphenyl)ethylcarbamoyl-(d)-argininylsarcosinylargininal



The compound of Example 130 (1.0 g) was hydrolyzed as described in Example 21 to give the title compound (0.39 g).

Example 132

Preparation of N-Boc-L-methionine sulfone-O-benzyl ester

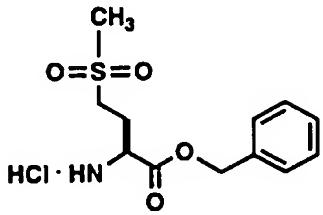


To a solution of N-Boc-L-methionine sulfone (50 g, 178 mmole) in dry THF (500 mL) which had been chilled to 0°C, carbonyl diimidazole (34.6 g, 214 mmole) was added in small portions. After 30 minutes, the mixture was warmed to room temperature for 2 hours until all of the CO_2 evolution ceased. After this time, benzyl alcohol (27.6 mL, 267 mmole) was added and the reaction stirred for 12 hours.

The reaction mixture was then reduced in volume under vacuum and the resulting residue was diluted with ethyl acetate (500 mL). The organic phase was then washed with saturated bicarbonate (1x100 mL), brine (100 mL), then saturated aqueous citric acid (1x100 mL), dried over $MgSO_4$, filtered and the solvent removed under vacuum to provide a white solid. The white solid was washed with a 1:1 mixture of diethyl ether/hexanes (300 mL) and filtered off on a Büchner funnel to provide 50.0g (92%) of the title compound. Thin layer chromatography analysis of the title compound showed a single spot with R_f=0.18 (silica, 3:2 hexanes/ethyl acetate).

Example 133

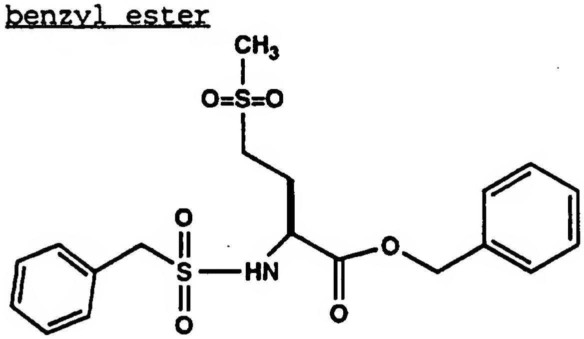
Preparation of L-methionine sulfone-O-benzyl ester hydrochloride salt



To the compound of Example 132 (50.0g), 200 mL of a 4M solution of HCl in dioxine was added. The solid eventually dissolved over 2 hours and showed no starting material by thin layer chromatography. The solution was then reduced in volume under vacuum and the resulting solid was washed with diethyl ether to provide 55.0 g (100%) of the title compound as a white solid.

Example 134

Preparation of N-benzylsulfonyl-L-methionine sulfone-O-benzyl ester



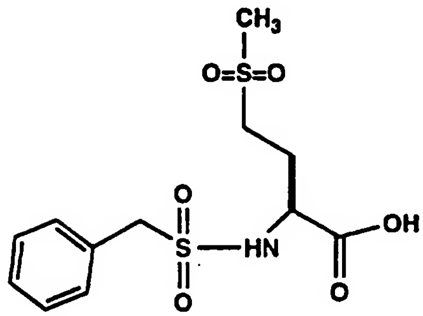
To a suspension of the compound of Example 133 (4.6 g, 15 mmole) in dry CH_3CN (35 mL) cooled to 0°C in an ice bath, benzylsulfonyl chloride (3.46 g) as a solution in CH_3CN (10 mL) is added, followed by pyridine (3.8 mL, 45 mmole). The reaction is allowed to stir in the ice bath for 15 hours while slowly warming to room temperature. The solvent is evaporated under vacuum to give a residue.

The resulting residue is dissolved in ethyl acetate (200 mL) and the solution is washed with saturated aqueous bicarbonate (50 mL), brine (50 mL), saturated aqueous citric acid (50 mL), and dried over $MgSO_4$. The solution is filtered and evaporated under vacuum to provide an oil. This crude product is purified by silica gel flash

chromatography to provide the title compound.

Example 135

Preparation of N-benzylsulfonyl-L-methionine sulfone



To a solution of the compound of Example 134 (1.6 g) in CH_3OH (50 mL), 250 mg 10% palladium on carbon is added. This mixture is then subjected to atmospheric hydrogenation at room temperature for 12 hours.. The reaction mixture is then filtered through a pad of celite and the solvent is evaporated under vacuum to provide the title compound.

Example A

Determination of IC_{50}.

The ability of the compounds of the present invention to act as inhibitors of factor Xa catalytic activity was assessed by determining the concentration which inhibited enzyme activity by 50%, (IC_{50}), using the purified human factor Xa.

The buffer used for all assays was HBSA (10 mM HEPES, pH 7.5, 150 mM sodium chloride, 0.1% bovine serum albumin).

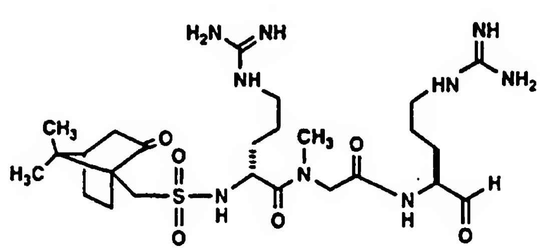
The assay was conducted by combining in appropriate wells of a Corning microtiter plate, 50 microliters of HBSA, 50 microliters of the test compound diluted in HBSA (or HBSA alone for uninhibited velocity measurement), and 50 microliters of the enzyme diluted in HBSA (prepared from purified human factor X obtained from Enzyme Research

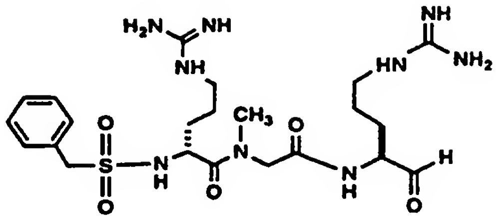
Laboratories according to the method described by Bock, P.E. et al. (1989) Archives of Biochem. Biophys. 273: 375. The enzyme was diluted into HBSA prior to the assay in which the final concentration was 0.5 nM). Following a 30 minute incubation at ambient temperature, 50 microliters of the substrate S2765 (N-alpha-benzyloxycarbonyl-D-argininyl-L-glycyl-L-arginine-p-nitroanilide dihydrochloride, obtained from Kabi Diagnostica and made up in deionized water followed by dilution in HBSA prior to the assay) was added to the wells yielding a final total volume of 200 microliters and a final concentration of 250 micromolar (about 5-times Km). The initial velocity of chromogenic substrate hydrolysis was measured by the change in absorbance at 405nm using a Thermo Max® Kinetic Microplate Reader over a 5 minute period in which less than 5% of the added substrate was utilized.

The concentration of added inhibitor which caused a 50% decrease in the initial rate of hydrolysis was defined as the IC_{50} value.

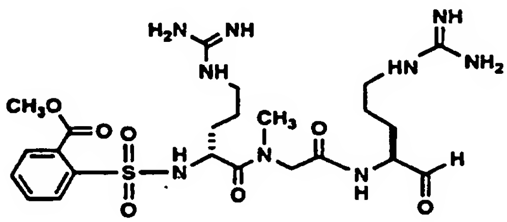
Table 1 shows the IC50 (nM) for certain preferred compounds (described in the noted Example) of the present invention.

Table 1. IC_{50} of Preferred Compounds.

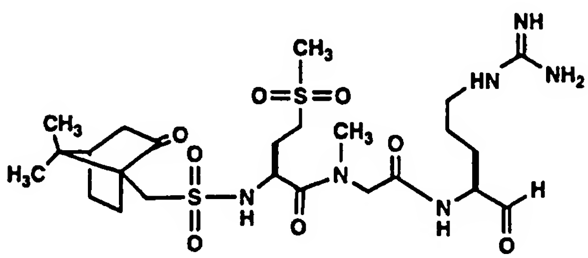
Compound	IC_{50} (nM)
	8.2 (Ex. 10)



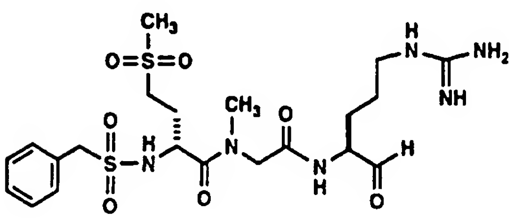
1.7 (Ex. 16)



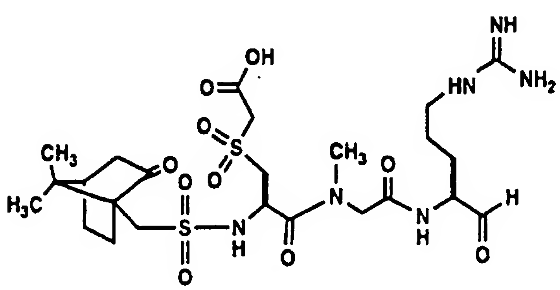
32 (Ex. 21)



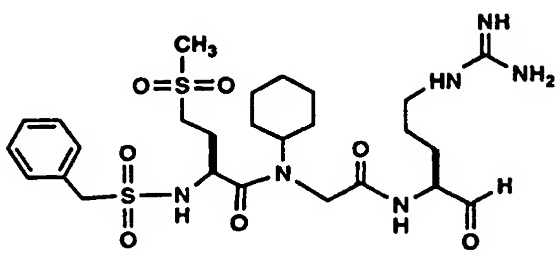
1.8 (Ex. 28)



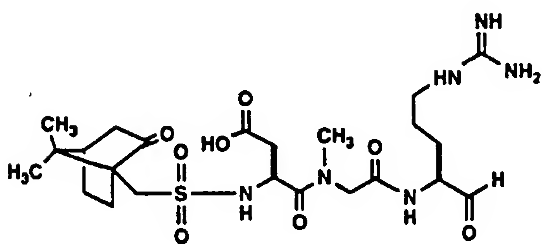
637 (Ex. 35)



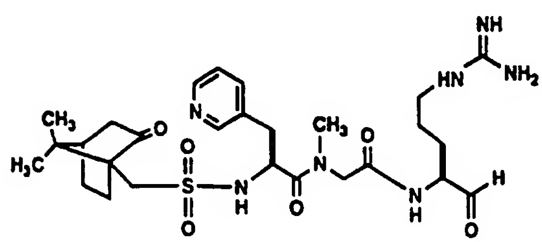
7.3 (Ex. 44)



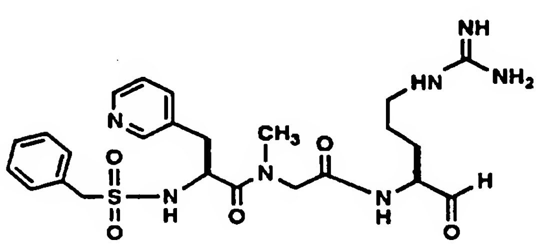
less than 25 (Ex. 50)



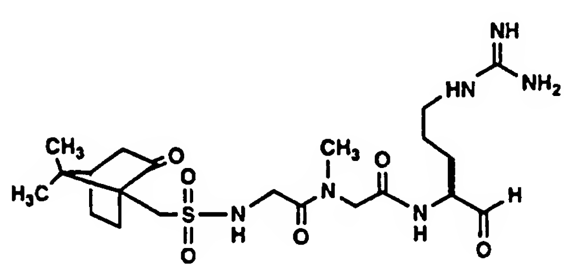
4.6 (Ex. 56)



101 (Ex. 62)



926 (Ex. 67)



614 (Ex. 73)

Example B

In vitro enzyme Assays for specificity determination.

The ability of compounds of the present invention to act as a selective inhibitor of factor Xa catalytic activity was assessed by determining the concentration of a certain compound which inhibited the activity of this enzyme by 50%, (IC_{50}), and comparing this value to that determined for all or some of the following related serine proteases: thrombin, recombinant tissue plasminogen activator (rt-PA), plasmin, activated protein C, chymotrypsin, and trypsin.

The buffer used for all assays was HBSA (10 mM HEPES, pH 7.5, 150 mM sodium chloride, 0.1% bovine serum albumin).

The assay for IC_{50} determinations was conducted by combining in appropriate wells of a Corning microtiter plate, 50 microliters of HBSA, 50 microliters of the test compound at a specified concentration (covering a broad concentration range) diluted in HBSA (or HBSA alone for V_o (uninhibited velocity) measurement), and 50 microliters of the enzyme diluted in HBSA. Following a 30 minute incubation at ambient temperature, 50 microliters of the substrate at the concentrations specified below, was added to the wells yielding a final total volume of 200 microliters. The initial velocity of chromogenic substrate hydrolysis was measured by the change in absorbance at 405nm using a Thermo Max® Kinetic Microplate Reader over a 5 minute period in which less than 5% of the added substrate was utilized. The concentration of added inhibitor which caused a 50% decrease in the initial rate of hydrolysis was defined as the IC_{50} value.

The factor Xa assay was conducted according to the procedure in Example A.

Thrombin Assay

Thrombin catalytic activity was determined using the chromogenic substrate Pefachrome t-PA (CH_3SO_2-D-hexahydrotyrosine-glycyl-L-arginine-p-nitroaniline,

obtained from Pentapharm Ltd.). The substrate was made up in deionized water followed by dilution in HBSA prior to the assay in which the final concentration was 300 micromolar (about 5-times Km). Purified human alpha-thrombin was obtained from Enzyme Research Laboratories, Inc. The enzyme was diluted into HBSA prior to the assay in which the final concentration was 0.25 nM.

Recombinant tissue plasminogen activator (rt-PA)

rt-PA catalytic activity was determined using the substrate, Pefachrome t-PA (CH_3SO_2-D-hexahydrotyrosine-glycyl-L-arginine-p-nitroaniline, obtained from Pentapharm Ltd.). The substrate was made up in deionized water followed by dilution in HBSA prior to the assay in which the final concentration was 500 micromolar (about 3-times Km). Human rt-PA (Activase®) was obtained from Genentech Inc. The enzyme was reconstituted in deionized water and diluted into HBSA prior to the assay in which the final concentration was 1.0 nM.

Plasmin Assay

Plasmin catalytic activity was determined using the chromogenic substrate, S-2251 [D-valyl-L-leucyl-L-lysine-p-nirtoanilide dihydrochloride], which was obtained from Kabi Diagnostica. The substrate was made up in deionized water followed by dilution in HBSA prior to the assay in which the final concentration was 300 micromolar (about 2.5-times Km). Purified human plasmin was obtained from Enzyme Research Laboratories, Inc. The enzyme was diluted into HBSA prior to assay in which the final concentration was 1.0 nM.

Activated Protein C (aPC)

aPC catalytic activity was determined using the chromogenic substrate, Pefachrome PC (delta-carbobenzyloxy-D-lysine-L-prolyl-L-arginine-p-nitroaniline dihydrochloride), obtained from Pentapharm Ltd.). The substrate was made up in deionized water followed by

dilution in HBSA prior to the assay in which the final concentration was 250 micromolar (about 3-times Km). Purified human aPC was obtained from Hematologic Technologies, Inc. The enzyme was diluted into HBSA prior to assay in which the final concentration was 1.0 nM.

Chymotrypsin

Chymotrypsin catalytic activity was determined using the chromogenic substrate, S-2586 (methoxy-succinyl-L-arginine-L-prolyl-L-tyrosyl-p-nitroanilide), which was obtained from Kabi Diagnostica. The substrate was made up in deionized water followed by dilution in HBSA prior to the assay in which the final concentration was 100 micromolar (about 9-times Km). Purified (3X-crystallized; CDI) bovine pancreatic alpha-chymotrypsin was obtained from Worthington Biochemical Corp. The enzyme was reconstituted in deionized water and diluted into HBSA prior to assay in which the final concentration was 1.0 nM.

Trypsin

Trypsin catalytic activity was determined using the chromogenic substrate, S-2222 (benzoyl-L-isoleucine-L-glutamic acid [gamma-methyl ester]-L-arginine-p-nitroanilide), which was obtained from Kabi Diagnostica. The substrate was made up in deionized water followed by dilution in HBSA prior to the assay in which the final concentration was 250 micromolar (about 4-times Km). Purified (3X-crystallized;TRL3) bovine pancreatic trypsin was obtained from Worthington Biochemical Corp. The enzyme was reconstituted in deionized water and diluted into HBSA prior to assay in which the final concentration was 0.5 nM.

The IC_{50} values (nM) for certain compounds according to this invention are shown in Table 2, and demonstrate the selectivity of compounds of this invention for Factor Xa compared to related serine proteases.

Table 2

Example	Xa	plasmin	PCa	tPA
10	8.16	1620	2500	>2500
16	1.1	777		
21	32	2500		
28	1.8	>2500	inactive	2500
35	637	2500	inactive	inactive
44	7.32	>2500	inactive	250-2500
50	13.4	>2500		
56	4.63	inactive	inactive	
62	101	inactive		
67	926	inactive	inactive	>2500
73	614	inactive	inactive	inactive
97A	25.5	1920		
97D	inactive	inactive	inactive	inactive
97E	3.42	1310	inactive	2500
97G	13.8	2.5-25	2500-2500	>2500
97H	>2500	>2500		
97I	22	>2500		
115C	4.22	>2500	inactive	>2500
115D	2.55	>2500	inactive	250-2500
115E	59.2	>2500	-	-
116D	10.3	>2500	-	-
116C	3.57	~2500	-	-
116F	10	>2500	-	-
117	4.56	4.33	-	-
118E	11.9	~2500	>2500	inactive
118I	36.9	>2500	>2500	inactive
118B	74.8	~2500	>2500	inactive
119B	8.06	1960	inactive	250-2500
120E	11.1	~2500	-	-
120H	9.6			
120G	2.8			
120B	20.4	>2500	-	-

120J	11.5			

- means not determined\

Example C

Ex vivo anticoagulant effects of (D)-camphorsulfonyl aspartyl sarcosine arginine aldehyde in human plasma.

The ex vivo anticoagulant effect of a compound of the present invention, (D)-camphorsulfonyl aspartyl sarcosine arginine aldehyde (Example 56), was determined by measuring the prolongation of the activated partial thromboplastin time (APTT) and prothrombin time (PT) over a broad concentration range of the added inhibitor, using pooled normal human plasma.

Fresh frozen citrated pooled normal human plasma was obtained from George King Biomedical, Overland Park, KA. The measurements for the APTT and PT were made using the Coag-A-Mate RA4 automated coagulometer (General Diagnostics, Organon Technica, Oklahoma City, OK) using Platelin® L or Simplastin® Excel (Organon Technica, Durham, NC) as the initiators of clotting, respectively, according to the manufacturers instructions. The assays were conducted by making a series of dilutions of the test compound in rapidly thawed plasma followed by adding either 200 microliters or 100 microliters to the wells of the assay carousel for the APTT and PT measurement, respectively.

As shown in Figure 2, (D)-camphorsulfonyl aspartyl sarcosine arginine aldehyde prolonged the APTT in a dose dependent manner in human plasma demonstrating an anticoagulant effect in humans. This would lead one skilled in the art to conclude that this compound will be an effective antithrombotic agent in humans.

Example D

Multiple Extracorporeal Shunt Model in Rats Utilizing Oral Dosing

The compound of Example 56 was evaluated in a multichamber A-V shunt model in rats. The A-V shunt model is one of the most common and generally used systems to evaluate antithrombotic compounds. Smith, J.R. and White, A.M. Br. J. Pharmacol., 77: 29-38 (1982). In this model a localized clot made up of primarily fibrin with some platelet and macrophage involvement (Shand, R. A. and Smith, J.R. and Wallis, R. B. Thromb. Res., 36: 223-232 (1984)), is formed on an artificial thrombogenic surface (typically a segment of silk or cotton thread) contained in a sialstic chamber which is part of an exteriorized shunt between the carotid artery and jugular vein. The procedure described in this Example is a modified A-V shunt model that allows for oral dosing of test agents and subsequent evaluation of efficacy over a two to three hour window in time.

Briefly, male Harlan Sprague Dawley rats (420-450 g) were acclimated at least 72 hours prior to use. The animals were fasted for 12 hours prior to surgery with free access to water. Unanesthetized animals were grouped into four dosage groups (six or seven animals per group) and administered test agents orally via gavage needle, at doses of 1.0, 3.0, 10 and 50 mg/kg. Immediately after oral dosing, animals were anesthetized with sodium pentobarbital (Nembutal) given intraperitoneally at a dose of 50 mg/kg body weight, and placed on a isothermal pad to maintain body temperature. The level of anesthesia was monitored every 15 minutes by neuro-response to a tail pinch, respiration and core temperature. The desired depth of surgical anesthesia was maintained by administering subsequent doses (5 mg/kg) intravenously. The left femoral artery was catheterized using standard procedures for blood pressure monitoring and blood sampling, with polyethylene tubing (PE50). The left femoral vein was catheterized with PE50 tubing for delivery of anethestic.

The exteriorized shunts were assembled by connecting two pieces of saline filled 12.5 cm PE90 tubing with a 6

cm piece of PE160 tubing containing a 6 cm piece of silk suture size 3 and clamped with hemostats. A small 0.5 cm portion of the silk thread protrudes from the junction of the chamber with the shunt. The left jugular vein and right carotid artery were catheterized with the ends of the PE90 shunt. The shunt was unclamped and blood allowed to flow from the carotid artery, through the chamber, and exits the shunt via the jugular vein. After 15 minutes, both sides of the chamber were clamped and the suture containing the clot removed following detachment of the arterial end of the chamber. The clot was immediately weighed and recorded. This procedure takes place at predetermined intervals (60, 90, 120, and 150 minutes after oral dosing) to allow assessment of efficacy over a large window in time. Four shunts were placed with flow initiated at 45, 75, 105, and 135 minutes after oral compound administration. Clot weight from the four shunts was the primary endpoint of the protocol. Blood pressure, heart rate core temperature and respiration were monitored continuously. Following termination of the experiment the animal was euthanized with a 120 mg/kg dose of Nembutal. One experiment was performed per animal.

Table 3 presents the data from this procedure, and demonstrates the oral activity of the compound of Example 56 in inhibiting thrombosis. At every dose tested, animals treated with the compound of Example 56 demonstrated smaller clots than animals treated with water. The compound's ability to decrease clot formation was maintained over time.

Table 3. Size of clot over time after oral dosing with test compound or water

	Clot size (mg) Minutes after oral dosing			
Oral dose	60 min	90 min	120 min	150 min
water	32.77	34.60	32.42	32.33

1.0 mg/kg	25.72	19.15	20.82	21.63
3.0 mg/kg	17.78	18.65	24.10	18.68
10 mg/kg	16.63	14.11	18.33	15.57
50 mg/kg	13.85	14.37	12.45	10.3

Example E

Evaluation of the antithrombotic activity of test compounds in the rat model of $FeCl_3$-induced platelet-dependent arterial thrombosis

The antithrombotic (prevention of thrombus formation) properties of compounds according to this invention were evaluated using an established experimental rat model of acute vascular thrombosis.

The rat $FeCl_3$ model is a well characterized model of platelet dependent, arterial thrombosis which has been used to evaluate potential antithrombotic compounds. Kurz, K. D., Main, B. W., and Sandusky, G. E., Thromb. Res., 60: 269-280 (1990). In this model a platelet-rich, occlusive thrombus is formed in a segment of the rat carotid artery treated locally with a fresh solution of $FeCl_3$ absorbed to a piece of filter paper. The $FeCl_3$ is thought to diffuse into the treated segment of artery and cause de-endothelialization of the affected vessel surface. This results in the exposure of blood to subendothelial structures which in turn cause platelet adherence, thrombin formation and platelet aggregation. The net result is occlusive thrombus formation. The effect of a test compound on the incidence of occlusive thrombus formation following application of $FeCl_3$ is monitored by ultrasonic flowtometry and is used as the primary end point. The use of flowtometry to measure carotid artery blood flow, is a modification of the original procedure in which thermal detection of clot formation was employed. Kurz, K. D., Main, B. W., and Sandusky, G. E., Thromb. Res., 60: 269-280 (1990).

Male Harlan Sprague Dawley rats (420-450 g) were acclimated at least 72 hours prior to use and fasted for

12 hours prior to surgery with free access to water. The animals were prepared, anesthetized with Nembutal followed by the insertion of catheters for blood pressure monitoring, drug and anesthesia delivery. The left carotid artery was isolated by making a midline cervical incision followed by blunt dissection and spreading techniques to separate a 2 cm segment of the vessel from the carotid sheath. A silk suture is inserted under the proximal and distal ends of the isolated vessel to provide clearance for the placement of a ultrasonic flow probe (Transonic) around the proximal end of the vessel. The probe is then secured with a stationary arm.

Following surgery the animals were randomized in either a control (saline) or treatment (compound of Example 56 or 16) group. The test compound (was administered as a single intravenous bolus at various doses after placement of the flow probe and 5 min prior to the thrombogenic stimulus. At t=0, a 3mm diameter piece of filter paper (Whatman #3) soaked with 10 µL of a 35% solution of fresh $FeCl_3$ (made up in water) was applied to the segment of isolated carotid artery distal to the flow probe. Blood pressure, blood flow, heart rate, and respiration were monitored for 60 minutes. The incidence of occlusion (defined as the attainment of zero blood flow) was recorded as the primary end point.

The efficacy of compounds of this invention as antithrombotic agents in preventing thrombus formation in this <u>in vivo</u> model was demonstrated by dose-dependent reduction in the incidence of thrombotic occlusion. The ED_{50} values for test compounds and for several well known anticoagulant agents in this model are shown in Table 4, below, and demonstrate the efficacy of the invented compounds.

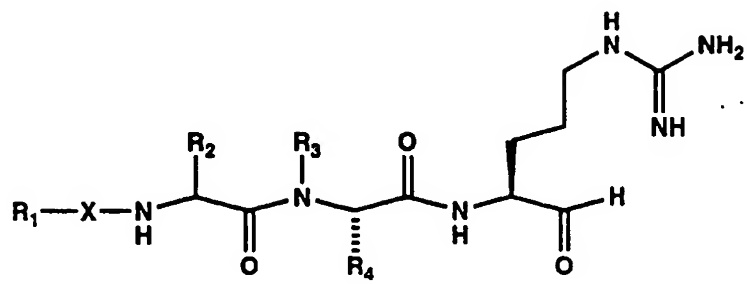
<u>Table 4</u>

Compound	ED50[a]
Standard Heparin	200 U/kg
Argatroban	3.8 mg/kg
Hirulog™	3.0 mg/kg
Compound Ex. 16	>5 mg/kg
Compound Ex. 56	4.5 mg/kg

[a]ED_{50} is defined as the dose that prevents the incidence of complete thrombotic occlusion in 50% of animals tested

WE CLAIM:

1. A compound of the formula:



wherein

(a) X is selected from the group consisting of $-S(O)_2-$, $-N(R')-S(O)_2-$, $-(C=O)-$, $-OC(=O)-$, $-NH-C(=O)-$, $-P(O)(R")-$ and a direct link, wherein R' is hydrogen, alkyl of 1 to about 4 carbon atoms, aryl of about 6 to about 14 carbon atoms or aralkyl of about 6 to about 16 carbon atoms, and R" is NR', OR', R', or SR', with the proviso that R" is not NH, OH, H, or SH, and ;

(b) R_1 is selected from the group consisting of:

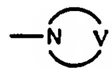
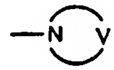
(1) alkyl of 1 to about 12 carbon atoms optionally substituted with Y_1,

(2) alkyl of 1 to about 3 carbon atoms substituted with cyclic alkyl of about 5 to about 8 carbon atoms optionally substituted on the ring with Y_1, Y_2 and/or Y_3,

(3) cyclic alkyl of 3 to about 15 carbon atoms, which optionally is substituted on the ring with Y_1, Y_2 and/or Y_3,

(4) heterocycloalkyl of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and $S(O)_i$, wherein i is 0, 1 or 2, optionally substituted on the ring with Y_1, Y_2 and/or Y_3,

(5) heterocyclo of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and $S(O)_i$, including

, wherein  is a 5 to 7 member heterocycle of 3 to 6 ring carbon atoms, where V is $-CH_2-$, -O-, -S(=O)-, $-S(O)_2-$ or -S-, and which is optionally substituted on the ring carbons with Y_1, Y_2 and/or Y_3,

(6) alkenyl of about 2 to about 6 carbon atoms which is optionally substituted with cyclic alkyl of about 5 to about 8 carbon atoms, which optionally is substituted on the ring carbons with Y_1, Y_2 and/or Y_3,

(7) aryl of about 6 to about 14 carbon atoms which is optionally mono-, di- or tri-substituted with Y_1, Y_2, and/or Y_3, respectively,

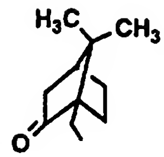
(8) heteroaryl of 5 to 14 atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and $S(O)_i$, and which is optionally mono-, di- or tri-substituted with Y_1, Y_2, and/or Y_3, respectively,

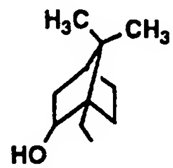
(9) aralkyl of about 7 to about 15 carbon atoms which is optionally substituted on the alkyl chain with hydroxy or halogen and mono-, di-, or tri-substituted in the aryl ring with Y_1, Y_2, and/or Y_3, respectively,

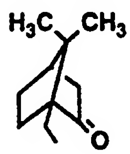
(10) heteroaralkyl of 6 to 11 atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and $S(O)_i$, and which is optionally substituted on the alkyl chain with hydroxy or halogen and optionally mono-, di- or tri-substituted on the ring with Y_1, Y_2, and/or Y_3, respectively,

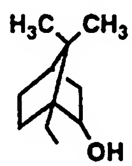
(11) aralkenyl of about 8 to about 15 carbon atoms which is optionally mono-, di-, or tri-substituted in the aryl ring with Y_1, Y_2, and/or Y_3, respectively,

(12) heteroaralkenyl of 7 to 12 atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and $S(O)_i$, and which is optionally mono-, di- or tri-substituted on the ring with Y_1, Y_2, and/or Y_3, respectively,

(13)  ,

(14)  ,

(15)  ,

(16)  ,

(17) difluoromethyl or perfluoroalkyl of 1 to about 12 carbon atoms,

(18) perfluoroaryl of about 6 to about 14 carbon atoms,

(19) perfluoroaralkyl of about 7 to about 15 carbon atoms, and

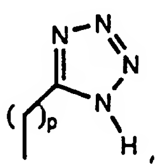
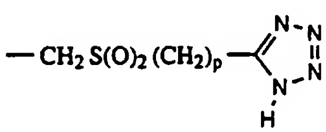
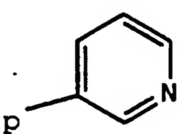
(20) hydrogen,

wherein Y_1, Y_2, and Y_3 are

(i) independently selected from the group consisting of halogen, cyano, nitro, tetrazolyl, amino, guanidino, amidino, methylamino, and methylguanidino, $-CF_3$, $-CF_2CF_3$, $-CH(CF_3)_2$, $-C(OH)(CF_3)_2$, $-OCF_3$, $-OCF_2CF_3$, $-OC(O)NH_2$, $-OC(O)NHZ_1$, $-OC(O)NZ_1Z_2$, $-NHC(O)Z_1$, $-NHC(O)NH_2$, $-NHC(O)NZ_1$, $-NHC(O)NZ_1Z_2$, $-C(O)OH$, $-C(O)OZ_1$, $-P(O)_3H$, $-P(O)_3H_2$, $-P(O)_3(Z_1)_2$, $-S(O)_3H$, $-S(O)_mZ_1$, $-Z_1$, $-OZ_1$, $-OH$, $-NH_2$, $-NHZ_1$, $-NZ_1Z_2$, and N-morpholino, wherein m is 0, 1 or 2, and Z_1 and Z_2 are independently selected from the group consisting of alkyl

of 1 to about 12 carbon atoms, aryl of about 6 to about 14 carbon atoms, heteroaryl of about 5 to about 14 atoms having 1 to about 9 carbon atoms, aralkyl of about 7 to about 15 carbon atoms, and heteroaralkyl of about 6 to about 11 atoms having about 3 to about 9 carbon atoms, or

(ii) Y_1 and Y_2 are selected together to be $-OC(Z_3)(Z_4)O-$, wherein Z_3 and Z_4 are independently selected from the group consisting of hydrogen, alkyl of 1 to about 12 carbon atoms, aryl of about 6 to about 14 carbon atoms, heteroaryl of about 5 to about 14 atoms having 1 to about 9 carbon atoms, aralkyl of about 7 to about 15 carbon atoms, and heteroaralkyl of about 6 to about 11 atoms having about 3 to about 9 carbon atoms, with the proviso that if X is not a direct link, then R_1 is not hydrogen,

(c) R_2 is selected from the group consisting of , , $-(CH_2)_p$, hydrogen, $-(CH_2)_pNHC(=NH)NH_2$, $-(CH_2)_pS(O)_2CH_3$, $-(CH_2)_pC(O)Z_5$, $-(CH_2)_pC(O)OZ_6$, $-(CH_2)_pC(O)NZ_6$, $-CH_2S(O)_2(CH_2)_pC(O)Z_5$, $-CH_2S(O)_2(CH_2)_pC(O)OZ_6$, $-CH_2S(O)_2(CH_2)_pC(O)NR_5R_6$, $-(CH_2)_pS(O)_2Z_6$, $-(CH_2)_pNH_2$, $-(CH_2)_pC(O)NR_5R_6$, $-(CH_2)_pC(O)Z_6$, $-(CH_2)_pOZ_6$ and $-(CH_2)_pC(O)-$ wherein

p is an integer from 1 to 6,

Z_5 is $-OH$, $-OCH_3$, $-OCH_2CH_3$, or $-NR_5R_6$,

Z_6 is alkyl of 1 to about 4 carbon atoms, aryl of about 6 to about 14 carbon atoms, or aralkyl of about 7 to 16 carbon atoms,

R_5 is hydrogen, or Z_6,

R_6 is hydrogen or cyclic alkyl of 3 to about 15 carbon atoms optionally mono- di- or tri-substituted with Y_1, Y_2 and/or Y_3, aralkyl of about 7 to about 15 carbon atoms optionally mono-, di- or tri-substituted with Y_1, Y_2 and/or Y_3, heteroaryl of 5 to 14 atoms with the ring atoms selected from carbon and heteroatoms wherein the

heteroatoms are selected from oxygen, nitrogen, and S(O)i and which is optionally mono-, di- or tri-substituted with Y_1, Y_2, and/or Y_3, quinuclidine, or adamantyl,

 is 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolinyl, 4-hydroxy piperidyl, 4-keto piperidyl, N-morpholino, 3,4-methylenedioxybenzyl piperazinyl, 4-phenyl piperazinyl optionally mono-substituted with fluoro, chloro, methoxy, or trifluoromethyl, or 4-benzyl piperazinyl optionally mono-substituted with fluoro, chloro, methoxy, or trifluoromethyl,

and pharmaceutically acceptable quaternary ammonium salts thereof;

(d) R_3 is selected from the group consisting of

(1) hydrogen;

(2) alkyl of 1 to about 8 carbon atoms optionally substituted with -OH;

(3) cyclic alkyl of about 3 to about 10 carbon atoms;

(4) alkyl of 1 to about 3 carbon atoms substituted with cyclic alkyl of about 5 to about 8 carbon atoms;

(5) aryl of about 3 to about 10 carbon atoms which is optionally mono-, di-, or tri-substituted with Y_1, Y_2 and/or Y_3;

(6) alkyl of 1 to about 3 carbon atoms substituted on the terminal carbon with aryl of about 4 carbon atoms to about 10 carbon atoms which is optionally mono-, di-, or tri-substituted with Y_1, Y_2 and/or Y_3;

(7) alkyl of 1 to about 6 carbon atoms with alkyl branching at the alpha, beta, gamma, and delta carbons of 1 to about 6 carbon atoms; and

(e) R_4 is selected from the group consisting of hydrogen, alkyl of 1 to about 7 carbon atoms optionally substituted with -OH or benzyloxy and alkyl of 1 to about 3 carbon atoms substituted on the terminal carbon atom with aryl of about 4 carbon atoms to about 10 carbon atoms

which is optionally mono-, di-, or tri-substituted with Y_1, Y_2 and/or Y_3.

2. A compound according to claim 1, wherein X is selected from the group consisting of $-SO_2-$, $-NH-S(O)_2-$, and $-N(R')-S(O)_2$.

3. A compound according to claim 2, wherein X is $-SO_2-$.

4. A compound according to claim 1, wherein R_1 is selected from the group consisting of alkyl, cycloalkyl, alkyl substituted with cyclic alkyl, aralkyl, and aryl groups all of which are optionally substituted.

5. A compound according to claim 4, wherein R_1 is selected from the group consisting of unsubstituted naphthyl, substituted naphthyl, nsubstituted phenyl, substittued phenyl, unsubstituted benzyl and substituted benzyl.

6. A compound according to claim 5, wherein R_1 is a substituted benzyl or a substituted naphthyl.

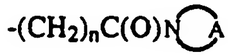
7. A compound according to claim 6, wherein R_1 is substituted with a substituent selected from the group consisting of $-C(O)OH$, $-C(O)OZ_1$, $-S(O)_mZ_1$, and $-CF_3$.

8. A compound according to claim 7, wherein the substituent is meta or ortho on the ring.

9. A compound according to claim 4, wherein R_1 is cyclohexyl or cyclohexylmethyl.

10. A compound according to claim 1, wherein R_2 is selected from the group consisting of $-CH_2CH_2CH_2NHC(=NH)NH_2$, $-CH_2CH_2S(O)_2CH_3$,

$-CH_2S(O)_2(CH_2)_nC(O)Z_3$, $-(CH_2)_nC(O)NR_5R_6$, and

.

11. A compound according to claim 10, wherein R_2 is selected from the group consisting of $-CH_2CH_2CH_2NHC(=NH)NH_2$, $-CH_2CH_2S(O)_2CH_3$, and $-(CH_2)_nC(O)NR_5R_6$.

12. A compound according to claim 11, wherein R_2 is $-CH_2CH_2CH_2NHC(=NH)NH_2$.

13. A compound according to claim 1, wherein R_5 is hydrogen.

14. A compound according to claim 1, wherein R_6 is selected from the group consisting of 3-(R)-quinuclidine, 3-(S)-quinuclidine, 4-trifluoromethyl-7-yl-coumarin, 4-methyl-7-yl-coumarin, 7-yl-coumarin, 3-yl-2-ethyl-4(3H)-quinazolinone, 2-yl-benzothiazole, 3-yl-benzoic acid, 3-yl-4-hydroxybenzoic acid, 4-hydroxy-1-methyl-6-phenyl-3-yl-2(1H)-pyridone, and 1-adamantyl, or ethyl morpholine, ethyl piperidine, 2-(2-ethyl)pyridine, 4-hydroxyphenethyl, (R)-alpha-methylbenzyl, (S)-alpha-methylbenzyl, 4-(methyl)-5-hydroxy-6-methyl-3-pyridine methanol, (1R,2S)-(N-methyl-N-(1-ethyl))benzyl alcohol, (1S,2R)-(N-methyl-N-(1-ethyl))benzyl alcohol, (1R,2R)-(N-methyl-N-(1-ethyl))benzyl alcohol, (1S,2S)-(N-methyl-N-(1-ethyl))benzyl alcohol, and 4-(methyl)-5-hydroxy-6-methyl-3-pyridine methanol.

15. A compound according to claim 1, wherein R_3 is selected from the group consisting of alkyl of 1 to about 7 carbon atoms optionally substituted with -OH on a terminal carbon atom, cyclohexylmethyl, phenyl, and benzyl.

16. A compound according to claim 15, wherein R_3 is

methyl or cyclohexyl.

17. A compound according to claim 16, wherein R_4 is hydrogen or alkyl of 1 to about 7 carbon atoms optionally substituted with -OH on a terminal carbon atom.

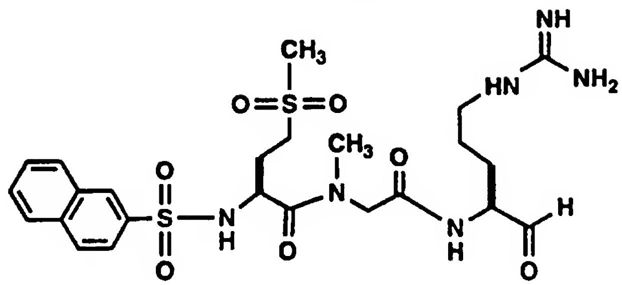
18. A compound according to claim 17, wherein R_4 is hydrogen.

19. A compound according to claim 1, wherein X is $-S(O)_2-$, R_1 is substituted or unsubstituted aralkyl, R_2 is $-CH_2CH_2CH_2NHC(=NH)NH_2$, R_3 is methyl, and R_4 is hydrogen.

20. A compound according to claim 1, wherein X is $-S(O)_2-$, R_1 is substituted or unsubstituted aralkyl, R_2 is $-CH_2CH_2NHC(=NH)NH_2$, R_3 is cyclohexyl, and R_4 is hydrogen.

21. A compound according to claim 20, wherein R_1 is substituted or unsubstituted benzyl.

22. A compound according to claim 1 selected from the group consisting of D-camphorsulfonyl aspartyl sarcosine arginine aldehyde, D-camphorsulfonyl cysteinesulfone-acetic acid sarcosine-arginine aldehyde, D-camphorsulfonyl-L-methionine sulfone sarcosine-arginine aldehyde, benzylsulfonyl-D-arginine-sarcosine-arginine aldehyde, (2-carbomethoxy)benzenesulfonyl-(D)-argininyl-sarcosine-argininal, N-benzylsulfonyl-(D)-methioninylsulfone sarcosine argininal, benzylsulfonylmethioninyl(sulfone)N-cyclohexylglycinylargininal, (D)-camphorsulfonyl aspartyl sarcosine arginine aldehyde, (D)-camphorsulfonyl-3-(3-pyridyl)-alanine sarcosine arginine aldehyde, benzylsulfonyl-3-(3-pyridyl)-alanine sarcosine arginine aldehyde, and (D)-camphorsulfonyl-glycine-sarcosine-arginine aldehyde,



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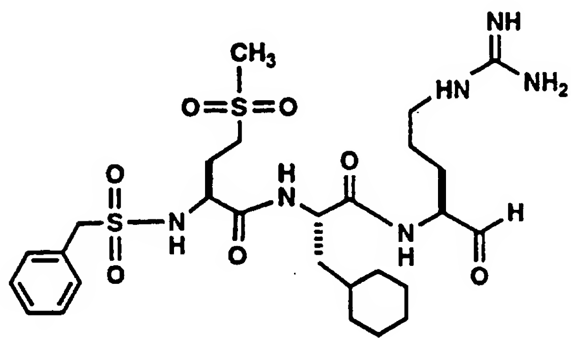

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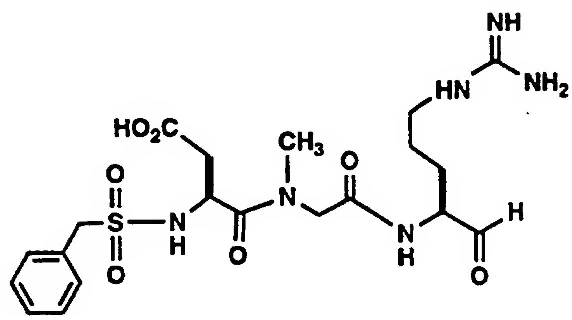

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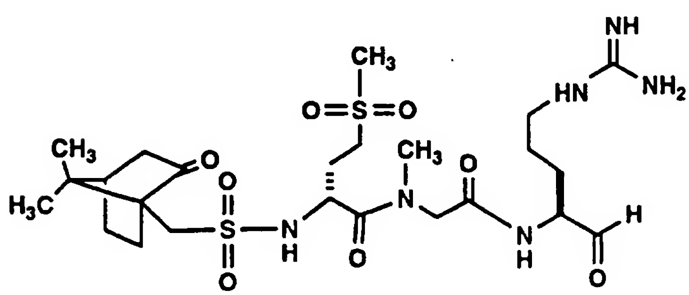

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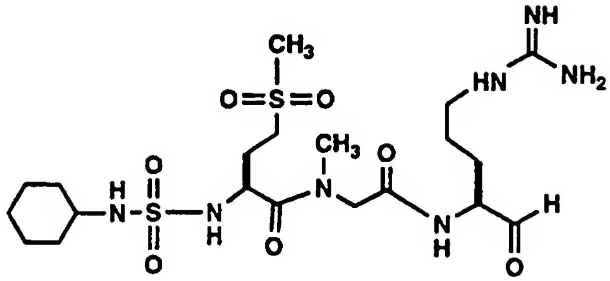

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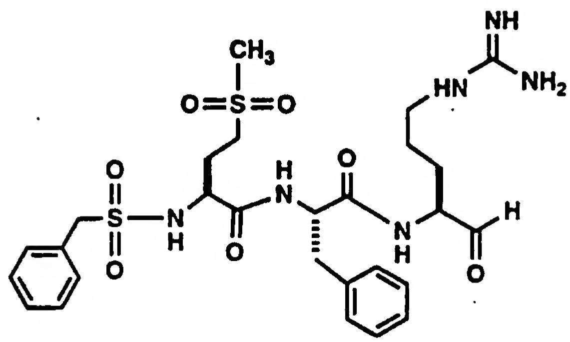

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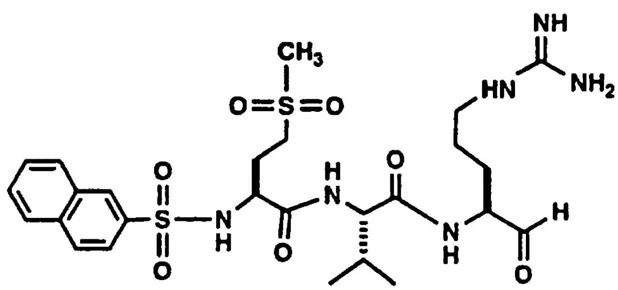

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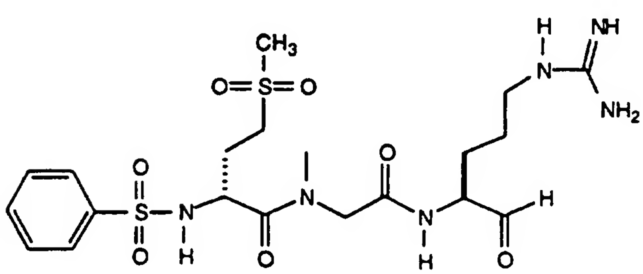
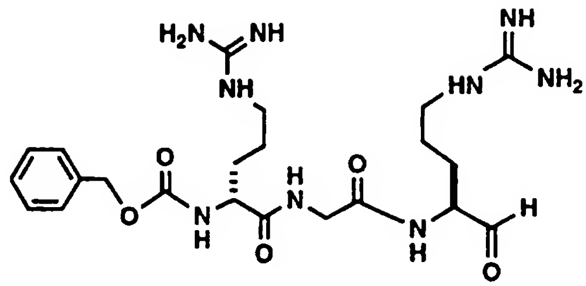


(h), and



(i).

23. A compound according to claim 1 wherein R_1 is mono-, di-, or tri-substituted with Y_1, Y_2 and/or Y_3 and Y_1, Y_2 and/or Y_3 are independently selected from -C(O)OH, -C(O)OZ_1, -S(O)$_m$$Z_1$, and -$CF_3$.

24. A compound according to claim 12 wherein X is -S(O)$_2$-.

25. A compound according to claim 24 wherein R_1 is alkyl, cyclic alkyl, alkyl substituted with cyclic alkyl, aryl or aralkyl, all of which are optionally substituted.

26. A compound according to claim 25 wherein R_1 is mono-, di-, or tri-substituted with Y_1, Y_2 and/or Y_3 and Y_1, Y_2 and/or Y_3 are independently selected from -C(O)OH, -C(O)OZ_1, -S(O)$_m$$Z_1$, and -$CF_3$.

27. A compound according to claim 25 wherein R_1 is unsubstituted naphthyl, substituted naphthyl, unsubstituted phenyl, substituted phenyl, unsubstituted benzyl or substituted benzyl.

28. A compound according to claim 27 wherein R_1 is benzyl.

29. A compound according to claim 25 wherein R_1 is cyclohexyl or cyclohexylmethyl.

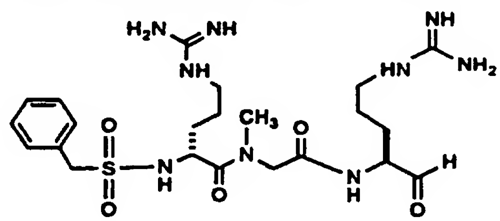
30. A compound according to claim 1 wherein R_2 is

$-CH_2CH_2S(O)_2CH_3$.

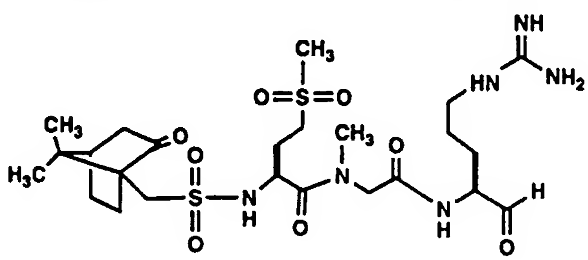
31. A compound according to claim 30 wherein X is $-S(O)_2-$ and R_1 is aralkyl.

32. A compound according to claim 31 wherein R_1 is mono-, di-, or tri-substituted with Y_1, Y_2 and/or Y_3 and Y_1, Y_2 and/or Y_3 are independently selected from $-C(O)OH$, $-C(O)OZ_1$, $-S(O)_mZ_1$, and $-CF_3$.

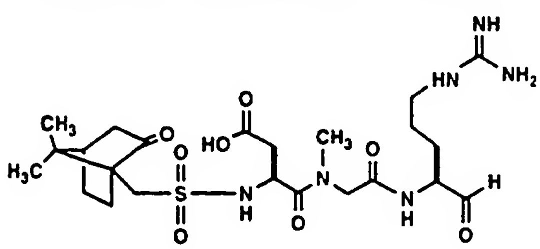
33. A compound according to claim 1 of the formula:



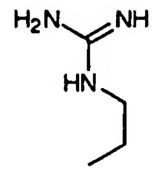
34. A compound according to claim 1 of the formula:



35. A compound according to claim 1 of the formula:



36. A compound according to claim 1 wherein X is

$-S(O)_2-$, R_1 is aralkyl and R_2 is , R_3 is methyl, cyclohexylmethyl, phenyl, or benzyl, and R_4 is hydrogen.

37. A compound according to claim 36 wherein R_1 is unsubstituted benzyl or substituted benzyl.

38. A compound according to claim 37 wherein Y_1 and Y_2 are independently selected from $-C(O)OH$, $-C(O)OZ_1$, $-S(O)_mZ_1$, and $-CF_3$.

Figure 1

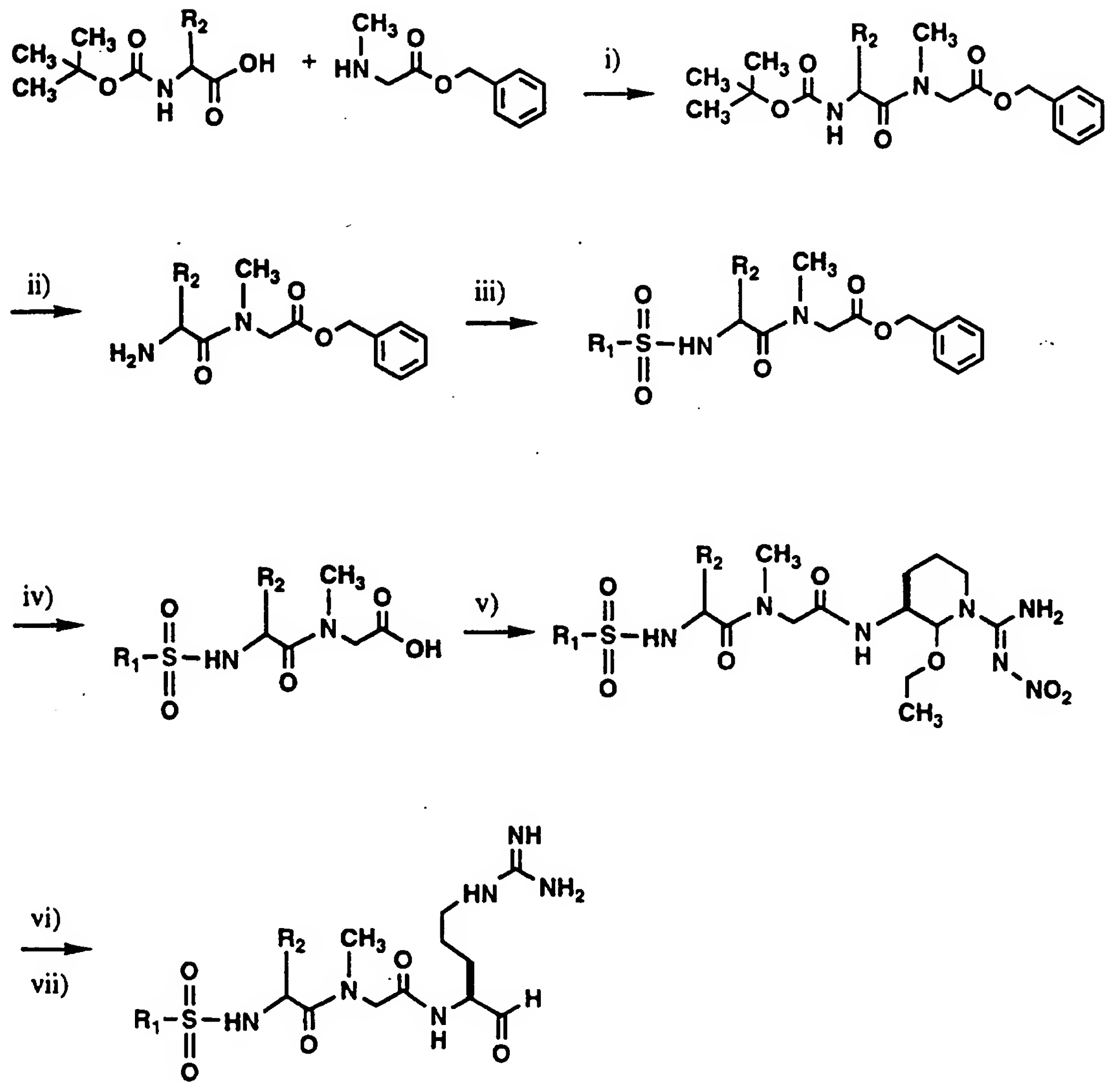


Figure 2

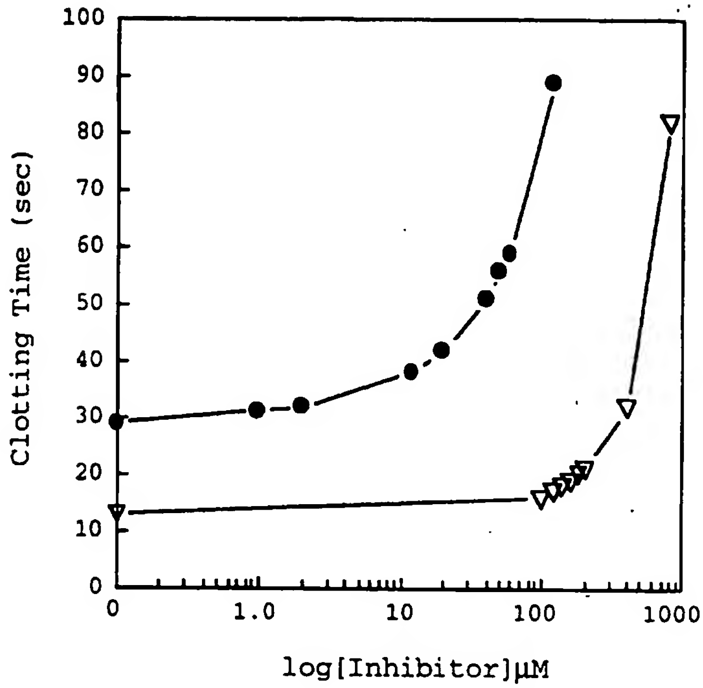

100
90
80
70
60
50
40
30
20
10
0
Clotting Time (sec)
0
1.0
10
100
1000
log[Inhibitor]µM

Figure 3

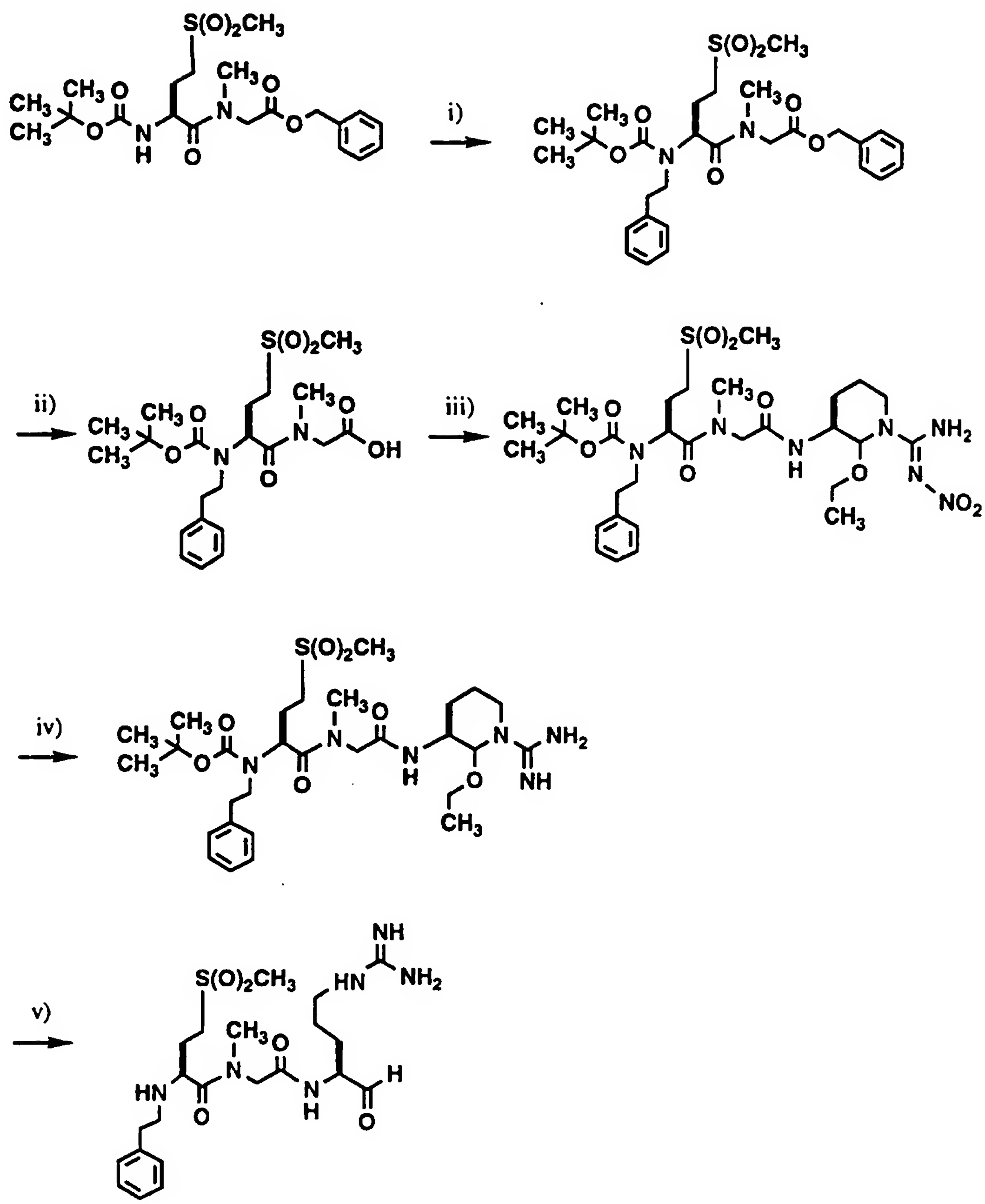


Figure 4

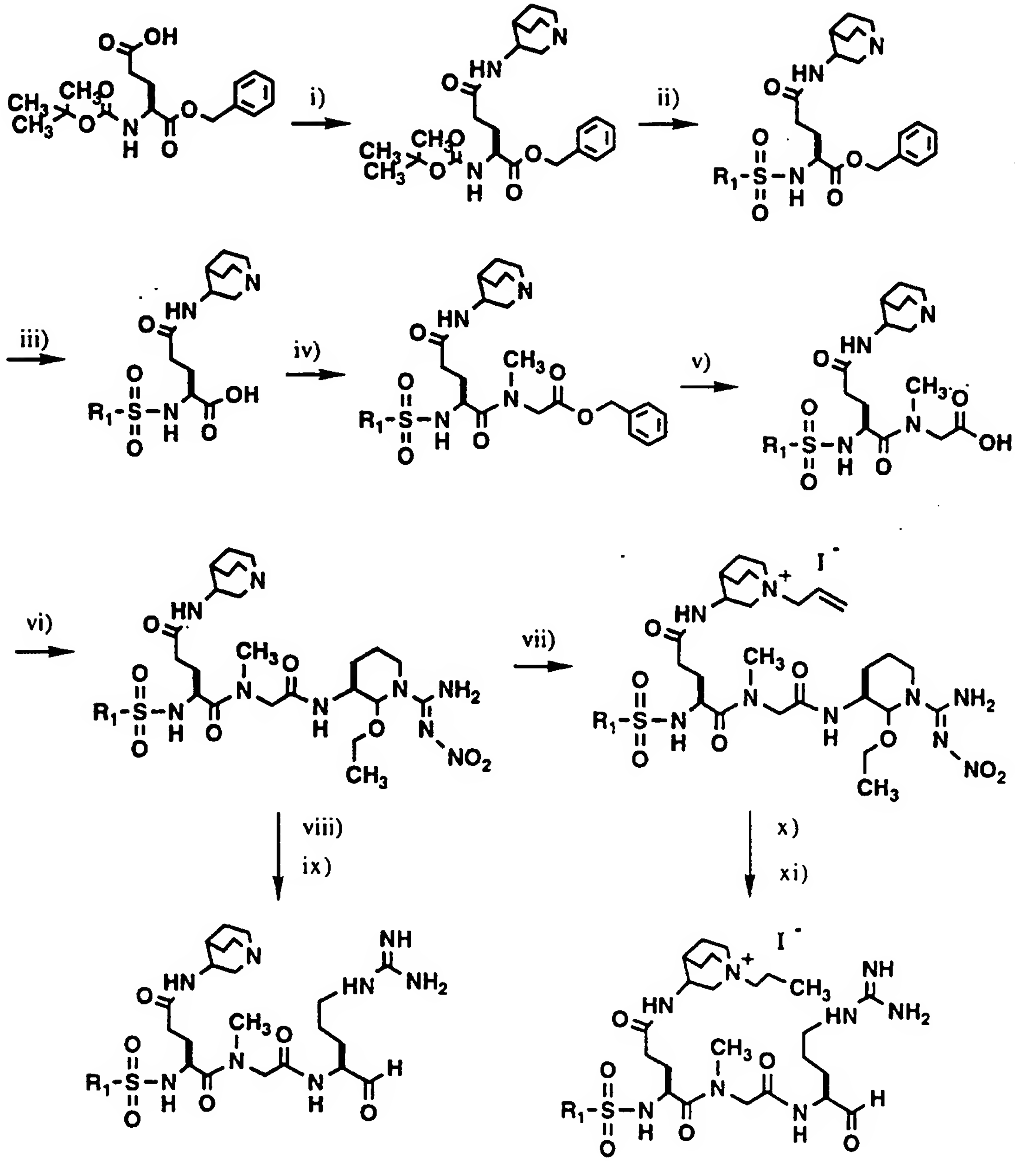
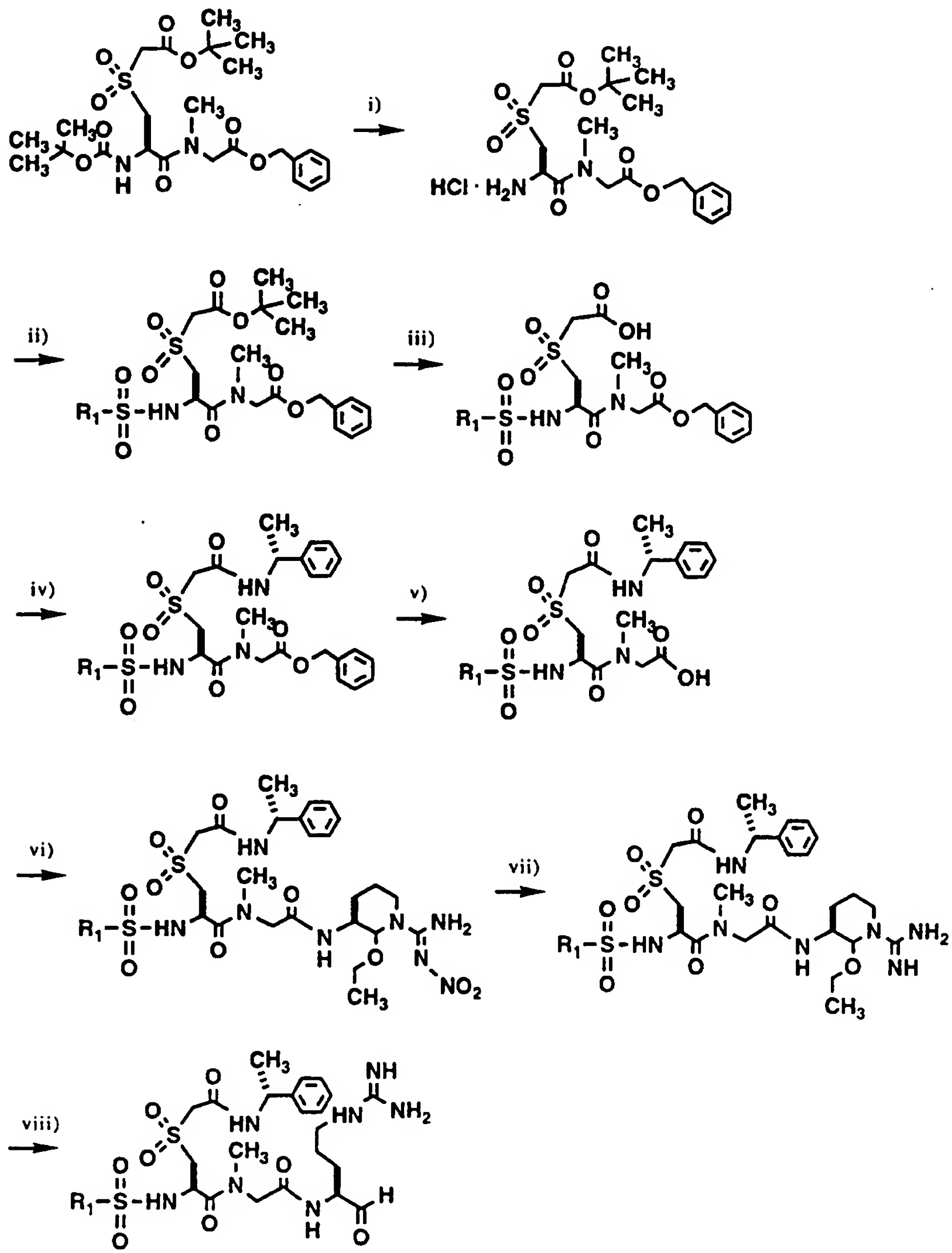


Figure 5



INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 95/16866

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K5/06 A61K38/55 //C07K14/81

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BRAZILIAN J. MED. BIOL. RES., vol. 27, 1994, pages 1943-1947, XP000566052 SZELKE, M. ET AL.: "Synthetic inhibitors of tissue kallikrein: ..." * page 1944, compound CH-851 * ---	1,4
X	WO,A,92 04371 (FERRING PEPTIDE RESEARCH PARTNERSHIP KB) 19 March 1992 * example 1; claim 1 * ---	1,4
A	EP,A,0 185 390 (RICHTER GEDEON VEGYESZETI GYAR R.T.) 25 June 1986 * pages 1-16 * ---	1
P,X	EP,A,0 672 659 (ELI LILLY &CO.) 20 September 1995 * claim 1; examples * ---	1-38

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
7 May 1996	23.05.96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hermann, R

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International Application No
PCT/US 95/16866

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,A	WO,A,95 28420 (CORVAS INT.,INC.) 26 October 1995 * abstract; claims * -----	1-38

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern. al Application No
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		JP-T- 6501461	17-02-94
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		AU-B- 5155385	26-06-86
		CA-A- 1261547	26-09-89
		CN-B- 1025738	24-08-94
		DE-A- 3584281	07-11-91
		JP-B- 6080078	12-10-94
		JP-A- 61152699	11-07-86
		SU-A- 1384203	23-03-88
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		JP-A- 7258287	09-10-95
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